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RHEUMATIC PNEUMONIA

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The matter of the occurrence of specific rheumatic pneumonia is unsettled at the present time, despite the fact that it has been studied thoroughly during the past decades. Uncertainty is indicated by the scarcity of information in textbooks, monographs and general reviews on rheumatic infection (Chiari [1]; Fahr [2]; Gräff [3]; Klinge [4]). During the course of histologic studies on the pulmonary alveolar lining, we observed interesting changes in the lungs of patients who had suffered from rheumatic infection. An investigation of the pulmonary material available in this department was undertaken to determine whether the pathologic characteristics of rheumatic infection in general could be found in the lung.

BRIEF REVIEW OF THE LITERATURE

We have restricted ourselves to the more recent papers, i. e., the papers published since 1937. Most of them make ample reference to previous publications.

Masson, Riopelle and Martin [5] examined 13 cases, the majority of which were in children between 4 and 11 years of age. Although they observed several types of alveolitis, particularly fibrinohemorrhagic alveolitis with mononuclear cell exudate, their most significant findings were hyaline lining membranes (in 10 cases) and peculiar granulomatous foci, which they called *bourgeons conjonctifs,* synonymous with connective tissue buds or sprouts (in 9 cases). The latter filled the alveolar ducts and were composed of a cellular granulation tissue which was different from that seen in other conditions. They expressed the belief that the rheumatic lung constituted a "specific anatomic entity."

Ravenna [6] described the case of a 14 year old girl with pneumonia complicating acute rheumatic carditis, polyarthritis and polyserositis. Fibrinoid swelling in the walls of the alveoli and the respiratory bronchioles, destruction of elastic fibers, proliferation of septal cells, edema and red infarction were the main findings. The genesis of the fibrinoid change was discussed. The writer stressed the clinical importance for the diagnosis of rheumatic pneumonia of acute pulmonary edema

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1. Chiari, H.: Die pathologische Anatomie des akuten Rheumatismus, Dresden, Theodor Steinkopff, 1938.
2. Fahr, T.: Ergebn. d. inn. Med. u. Kinderh. **54**:357, 1938.
3. Gräff, S.: Rheumatismus und rheumatische Erkrankungen, Berlin, Urban & Schwarzenberg, 1936.
4. Klinge, F.: Ergebn. d. allg. Path. u. path. Anat. **27**:1, 1938.
5. Masson, P.; Riopelle, J. L., and Martin, P.: Ann. d'anat. path. **14**:359, 1937.
6. Ravenna, P.: Minerva med. **2**:420, 1937.

and dyspnea arising in the course of rheumatic fever and not referable to cardiac insufficiency.

Hadfield [7] studied 5 cases and found widespread fibrinous alveolitis, which was followed in later stages by mononuclear cell infiltration. He saw hyaline lining membranes and fibrinous exudate in the alveolar ducts and noted that large numbers of tributary alveoli were sealed off by this material. Hadfield felt that granulomatous lesions such as those described by Masson and his co-workers developed later by organization of this fibrinous exudate.

Gouley [8] examined the lesions of the lungs in rheumatic fever with particular regard to their evolution in chronic stages. He described interstitial pneumonitis that began in foci of fibrinoid necrosis in the alveolar walls. This was accompanied by monocytic infiltration. Later Aschoff cells were present, and, finally, diffuse interstitial fibrosis with hyperplasia of elastic tissue resulted. The lesions were not directly dependent on passive congestion or mitral stenosis.

Gamna [9] expressed the belief that the question of the occurrence of rheumatic pneumonia was unanswered as far as the pathologic changes were concerned. However, he recalled clinical instances in which fleeting, often reversible exudative processes occurred in the lungs and in which salicylates were employed successfully. He considered the possibility that brown induration of the lungs might result from interstitial sclerosis of a specific rheumatic nature.

Nittono and Hoshiyama [10] observed rapidly spreading atypical lobar pneumonia in the course of acute rheumatic polyarthritis. The bacteriologic findings were negative, and sulfonamide compounds were useless. Necropsy disclosed hemorrhagic pneumonia with a rubbery consistency of the lungs. Microscopic examination revealed arteriolitis, capillary swelling, fibrinoid degeneration of the alveolar walls, serofibrinous exudate, monocytes and desquamated alveolar lining cells.

Massa [11] observed clinical features similar to those pointed out by Gamna [9] and emphasized the negative bacteriologic findings.

Epstein and Greenspan [12] concluded from their studies that specific rheumatic pneumonia does not occur. They never found Aschoff bodies in the lungs in their series of 45 cases. However, they did observe a characteristic, though not specific, microscopic process that consisted of alveolitis, congestion, edema and the formation of hyaline lining membranes. These lesions were interpreted as due to alterations in the capillaries.

MATERIAL

Sixty-three consecutive cases of active and quiescent rheumatic fever were examined pathologically. Eight cases showed distinctive microscopic pulmonary changes; in this group most of the necropsies had been performed by one of us. Sixty cases were used as controls at the beginning of the study: 20 cases of various types of acute pneumonia; 20 cases of uncomplicated chronic passive congestion of the lungs; 10 cases of chronic passive congestion complicated by ordinary pneumonia; 10 cases of chronic organizing pneumonia. This original control series was expanded as appropriate current material became available.

REPORT OF CASES

Case 1.—D. N., a white boy 14 years old, entered Colorado General Hospital with a history of repeated attacks of pain in the chest and heart trouble in the preceding eight years, shortness of breath for two years, cough and bloody sputum for three days. The previous past personal history and the family history were not remarkable. Physical examination

7. Hadfield, G.: Lancet **2**:710, 1937.
8. Gouley, B. A.: Am. J. M. Sc. **196**:1, 1938.
9. Gamna, C.: Med. Klin. **36**:122, 1940.
10. Nittono, F., and Hoshiyama, J.: Jap. J. M. Sc., V, Path. **5**:315, 1940.
11. Massa, R. G.: Rev. méd. de Córdoba **29**:667, 1941.
12. Epstein, E., and Greenspan, E.: Arch. Int. Med. **68**:1074, 1941.

revealed signs of aortic valvular insufficiency, i. e., a Corrigan pulse, blood pressure 150 systolic and 0 diastolic, pulsation of the capillary bed and characteristic heart murmurs. The lung fields were moist; crackling rales were audible bilaterally.

The patient was placed in an oxygen tent, digitalized and given sulfathiazole. The rectal temperature varied from 99.2 to 102.6 F.; the pulse rate, from 100 to 120; the respiratory rate, from 28 to 60, mainly around 40. The white blood cell count on admission was 24,760, with 80 per cent polymorphonuclears; six days later it rose to 42,520 with 83 per cent polymorphonuclears. Death occurred one week after admission.

Necropsy yielded the following diagnosis: rheumatic heart disease, i. e., diffuse pancarditis (pericarditis, myocarditis, endocarditis), active, severe, with decompensation; cardiac hypertrophy; severe bilateral confluent lobular pneumonia; chronic passive congestion of the liver, lungs, spleen, gastrointestinal tract and kidneys.

The right lung weighed 865 Gm.; the left, 820 Gm. Both organs showed similar changes. The visceral pleura revealed scattered fibrous adhesions, some interlobar. The external surfaces of the lungs were deep purple; the consistency was solid and noncrepitant except for a narrow crepitant fringe anteriorly. On section the surfaces were deeply congested, bloody and mottled purplish red. The bronchi and the blood vessels were not remarkable.

Microscopically, the lungs displayed extensive hyaline lining membranes in the alveolar ducts and alveoli. Scattered small inflammatory foci were apparent in the acini, with fibrinoid necrosis of the alveolar walls. The foci appeared to be peppered with polymorphonuclears and lymphocytes and contained extravasated erythrocytes and many small pyknotic and fragmented nuclei. In addition, fibrinoid degeneration of the walls of blood vessels, some of which were infiltrated by polymorphonuclears, was noted. The septal cells were swollen, and many alveoli contained foamy phagocytes. Numerous distinctive granulomatous lesions were observed: Some were composed of masses of fibrin located within the alveolar ducts or the alveoli themselves; others consisted of spindle-shaped fibroblasts, polymorphonuclears, pigmented phagocytes, cells resembling plasma cells except for eosinophilic cytoplasm, erythrocytes and small strands or clumps of fibrin. Cuboidal cells were scattered or arranged in short rows on the surface, but there was no complete covering.

Case 2.—J. G., a white boy 13 years old, entered Colorado General Hospital with the complaints of pain in the chest and cough of five days' duration and "heart trouble" for the past two years. The temperature was 101.0 F.; the pulse rate, 120; the respiratory rate, 40, and the blood pressure 78 systolic and 50 diastolic. Respirations were shallow and rapid, and there was moderate cyanosis. Auscultation of the chest disclosed an increase in breath sounds in the right basal and axillary regions. The heart was enlarged to the left anterior axillary line in the fourth intercostal space. Loud systolic and diastolic murmurs were audible over the precordium and extended into the left axilla.

The white blood cell count was 8,320, with 82 per cent polymorphonuclears, 15 per cent lymphocytes and 3 per cent eosinophils. A roentgenogram of the chest on admission demonstrated cloudiness over the lower two thirds of the right lung. This was interpreted as extensive pneumonia. Roentgen examination five days later revealed considerable clearing of the right lung.

The patient was placed in an oxygen tent, digitalized and given sulfathiazole. The clinical course was downhill and of a septic character: The temperature fluctuated between 101.0 and 103.0 F. Increasing restlessness and dyspnea developed; death occurred nine days after admission.

Necropsy disclosed: severe acute rheumatic pancarditis (fibrinohemorrhagic generalized pericarditis, diffuse myocarditis, mitral endocarditis with secondary stenosis), with decompensation; severe bilateral confluent lobular pneumonia, chronic passive congestion of the liver, lungs, spleen, gastrointestinal tract and kidneys.

The right lung weighed 750 Gm.; the left, 575 Gm. The changes were essentially the same in both organs. The visceral pleura appeared thin and transparent, and the lungs were dark purple over all aspects except for small scattered pink patches of normal tissue. In general consistency each lung was firmly consolidated and liver-like except in the normal pink areas. On section the surfaces oozed abundant frothy bloody fluid and showed diffuse red congestion with scattered normal zones. The bronchi and the blood vessels were not remarkable.

Microscopically, the lungs revealed intense hyperemia of medium and small vessels. The alveolar walls were congested and exhibited focal fibrinoid degeneration, which in some instances involved small arterial branches. Most foci were small; a few were confluent and displayed involvement of groups of as many as twelve alveoli. The arteriolar walls were swollen, fibrinous and infiltrated by polymorphonuclears and lymphocytes, which dispersed

into the perivascular tissue. In these zones many nuclei were pyknotic and fragmented. Some vessels were surrounded by extravasated red cells. Several small arteries showed fibrinoid swelling. Many intervening alveoli exhibited diffuse acute inflammation of low grade and contained thin strands of fibrin, polymorphonuclears, red cells and phagocytes; the alveolar capillaries were hyperemic, and the septal cells were swollen. In one or two zones the alveoli were plugged with thick masses of fibrin and enmeshed polymorphonuclears. Many small bronchioles showed congestion and focal fibrinoid necrosis in the submucosa, with inflammatory changes similar to those around the arterioles.

CASE 3.—F. T., a white girl 12 years old, entered Colorado General Hospital for the last time one month before death. She had a history of heart disease and repeated attacks of rheumatic fever for the past six years. At this last admission she complained of vomiting, sore throat, nosebleed and pain in the joints for the past several weeks. The temperature was 103.6 F.; the pulse rate, 120; the respirations, 36; the blood pressure, 110 systolic and 55 diastolic. The mucous membranes of the throat were pale, in part injected. The chest presented symmetric excursions, and there was questionable dulness over the right posterior aspect. Bronchial breathing and scattered rales were audible on the right. The heart was greatly enlarged, and an apical thrust was palpable 10.5 cm. to the left of the midline in the sixth interspace. Loud systolic and diastolic murmurs were heard over the apex.

The white blood cell count was 14,700, with 88 per cent polymorphonuclears; the red cell count was 3,980,000. A roentgenogram of the chest on admission showed nothing abnormal.

The temperature was of the septic type, spiking to 104.0 F. daily. The patient complained of generalized aches. There was no clinical evidence of pneumonia. Treatment consisted of rest in bed, administration of vitamins, iron, acetylsalicylic acid, sulfathiazole and digitalis, and blood transfusion.

At necropsy the following conditions were observed: chronic rheumatic pancarditis (pericarditis, myocarditis, endocarditis with mitral stenosis), with decompensation; cardiac hypertrophy and dilatation; bilateral hemorrhagic confluent lobular pneumonia; subacute ulcerative esophagitis.

The right lung weighed 460 Gm.; the left, 295 Gm. The visceral pleura was smooth but appeared somewhat dull. The left lung showed bluish red mottling externally and was of nodular consistency; the right was unformly dark red, heavy, and firm to palpation. The surface of the right lung on section was dark red, dry, slightly granular and noncrepitant; the infiltration diminished in scattered patches in the anterior portion of the upper lobe, where part of the lung tissue was intact, grayish pink and crepitant. The cut surface of the left lung revealed patchy, dark red areas of consolidation. The arteries and the bronchi were not noteworthy.

Microscopically, the parenchyma exhibited diffuse congestion, and many alveoli were filled with extravasated red cells. Large foci of fibrinoid necrosis were observed: the alveolar walls showed eosinophilic fibrinoid swelling and were infiltrated by polymorphonuclears. The alveolar lumens contained fibrin, red cells, polymorphonuclears, foamy macrophages, pyknotic nuclei and cellular debris. Vascular changes were not prominent. Some alveoli and ducts were plugged with fibrin and red cells. Hyaline lining membranes were present in occasional fields. Some of the small bronchioles contained plugs of fibrin mixed with mucus and red cells.

CASE 4.—V. V. S., a white woman 22 years old, was admitted to Colorado General Hospital one week before death. During the previous two years she had been hospitalized on several occasions for nephritis. She gave a history of periodic shortness of breath, generalized weakness and nocturia for three years. Repeated attacks of tonsillitis had occurred; no history of scarlet or rheumatic fever was obtained. At the time of the last entry she complained of nosebleed, shortness of breath, vomiting and headache of four days' duration. The temperature was 101.5 F.; the pulse rate, 92; the respiratory rate, 26; the blood pressure 205 systolic and 165 diastolic. The lungs were not remarkable. The heart was moderately enlarged to the left, with gallop rhythm, and there was a systolic murmur over the apex. The eyegrounds showed bilateral albuminuric retinitis and moderate papilledema.

On admission the white blood cell count was 6,180, with 88 per cent polymorphonuclears; the red cell count was 2,590,000. Later the white cell count rose to 17,400, with 90 per cent polymorphonuclears. Urinalysis disclosed albumin, granular casts and pus cells. The nonprotein nitrogen of the blood amounted to 168 mg. per hundred cubic centimeters. A roentgenogram of the chest demonstrated infiltration in the lower three fourths of both lungs; the changes were in the form of fine stippling and mottling and resembled miliary tuberculosis to some degree.

The patient was given fluids, oxygen and sulfadiazine but without improvement. The temperature rose to 101.6 F.; the respirations became labored and their rate rose to 44; terminally, convulsions and coma developed.

At necropsy the following diagnosis was made: chronic glomerulonephritis with uremia; rheumatic heart disease with mitral insufficiency; cardiac hypertrophy; bilateral lobular pneumonia.

The right lung weighed 912 Gm.; the left, 855 Gm. Both lungs were pale purple-gray; all lobes except the right middle one displayed infiltration and consolidation. On section the surfaces were reddish purple and oozed frothy fluid; similar material was present in the smaller bronchi.

Microscopically, minute and large foci of fibrinoid necrosis were seen; even the largest were less than lobular in size. In these foci peppering by polymorphonuclears and the presence of extravasated red cells, pyknotic nuclei and nuclear fragments were noted. Septal cell swelling and lining of alveoli by septal cells were prominent. In many alveoli there were marked fibrin deposits, sometimes in masses that filled the alveoli or the ducts; the adjacent alveoli displayed septal cell proliferation and lining. Formation of hyaline membranes was marked. Edema, foamy phagocytes and hyperemia were observed in intervening alveoli. Vascular changes were absent.

CASE 5.—R. H., a white man 52 years old, was admitted to Colorado General Hospital eleven days before death. He complained of pain in the stomach, shortness of breath of one month's duration and pain over the right side of the chest during the last three weeks. His latest attack of pain in the joints had occurred five years previously. The temperature was 98 F.; the pulse rate, 92; the respiratory rate, 32; the blood pressure, 160 systolic and 90 diastolic. The patient was dyspneic; bronchial breathing was audible in both axillas. The heart rate was markedly irregular; systolic and diastolic murmurs were heard. Pitting edema was present in the lower extremities.

A roentgenogram of the chest demonstrated pulmonary congestion with homogeneous density in the left middle third. The white blood cell count was 14,280, with 80 per cent polymorphonuclears. Urinalysis disclosed albumin (2 plus), pus (2 plus) and red cells (1 plus).

The patient was given digitalis; this treatment was stopped later and the digitalis replaced by quinidine. The temperature rose to 102 F, rectally, and the respiratory rate increased to 40. Sulfathiazole was administered for possible bronchopneumonia and was supplemented by an oxygen tent. A subsequent roentgenogram showed some clearing of the lungs, but the clinical course was downhill, and death took place a few days later.

At necropsy the following conditions were observed: rheumatic heart disease, with severe aortic stenosis and moderate mitral endocarditis; extreme cardiac hypertrophy to the left (1,050 Gm) and dilatation; chronic passive congestion of the lungs.

No weights were recorded for the lungs. The pleura showed old fibrous adhesions. Otherwise the external surfaces of the lungs were smooth and purplish gray and to palpation were crepitant anteriorly and subcrepitant posteriorly. On section the surfaces of the left lung were wet and rubbery, with purplish gray mottling, and were slightly granular. The right lung was similar except for extensive purplish red consolidation in the upper lobe and smaller similar zones in other parts.

Microscopically, small and large foci of granulation tissue were apparent in the lumens of alveoli and ducts. The small foci were composed of fibroblasts and capillaries; some were papilliform and projected into the lumens. The larger ones consisted of fibroblasts, capillaries, red cells, free and phagocytosed pigment, lymphocytes and polymorphonuclears. Elsewhere the capillaries were congested and the alveoli contained scattered polymorphonuclears and pigmented phagocytes. Some alveoli were filled with red cells or fresh fibrin. Septal cell swelling and proliferation were marked. The larger arteries displayed mild to moderate hyaline thickening; the smaller ones showed endothelial swelling and occasional polymorphonuclears in the wall.

CASE 6.—I. B., a white girl 12 years old, entered Colorado General Hospital with a history of having had rheumatic fever since the age of 5 years. An attack of chorea of three weeks' duration was experienced at that time; another attack occurred one year later. At the age of 10 years an episode of sore throat with swelling of the joints and dyspnea persisted for six weeks. The present illness began with sore throat, ten days before hospitalization; one week later headaches, loss of appetite, fever, fatigue and weakness developed. The toes became painful, and shortness of breath appeared one day before admission. The temperature was 103.2 F.; the pulse rate, 120; the respiratory rate, 54; the blood pressure, 90 systolic and 50 diastolic. The tonsils and the pharynx were congested. The apex beat was found in

the fourth interspace, 9 cm. to the left of the midsternal line. A systolic murmur was audible over the apex. The toes and the ankles were tender.

The white blood cell count was 28,950, with 80 per cent polymorphonuclears.

On the next day symptoms of pulmonary congestion developed, with cough and expectoration of blood-tinged mucus. A roentgenogram of the chest demonstrated diffuse congestive changes throughout both lungs. The temperature continued to be elevated. The patient was placed in an oxygen tent. Two days after admission she died.

At necropsy the following diagnosis was made: rheumatic pancarditis (endocarditis with mitral insufficiency and stenosis, myocarditis, acute pericarditis); cardiac hypertrophy and dilatation; pulmonary congestion.

The right lung weighed 755 Gm.; the left, 645 Gm. The pleura was dull, with traces of thin fibrinous coating. The consistency of the lungs was subcrepitant. On section the surfaces were bluish red, congested and moist. There were no zones of infiltration or hemorrhage.

Microscopically, small foci of fibrinoid necrosis were found in the alveolar walls. The lesions frequently involved small vessels and were associated with capillary hyperemia. Peppering by polymorphonuclears, extravasation of red cells and presence of scanty hyaline membranes, foamy macrophages, swollen septal cells and traces of septal cell lining were observed in these foci.

Case 7.—W. M., a white boy 16 years old, first became ill with severe sore throat, followed by intermittent fever, which continued for several months, and pain in the lower part of the back. There was no past history of rheumatic infection, and the heart was considered normal on repeated examinations. Three months later, excruciating pain developed in the left upper abdominal quadrant. The boy was then admitted to the Children's Hospital. He was suffering from marked shortness of breath and was placed in an oxygen tent. The temperature was 101.0 to 103.0 F. At this time systolic and diastolic murmurs first became audible, particularly over the aortic area. The blood pressure was 170 systolic and 100 diastolic. Cough was absent.

The white blood cell count was 27,100, with 91 per cent polymorphonuclears; later it varied between 19,000 and 27,300, with an average of 90 per cent polymorphonuclears. A roentgenogram of the chest demonstrated enlargement of the heart and congestion in the right lung.

The course was rapidly downhill, and the patient died less than three weeks after the onset of the abdominal pain.

Necropsy disclosed the following conditions: severe, acute rheumatic pancarditis (endocarditis of the mitral, aortic and tricuspid valves, myocarditis, partially organized acute, fibrinous pericarditis); extensive bilateral pneumonia; marked congestion of viscera.

The right lung weighed 720 Gm.; the left, 705 Gm. The pleura was covered with fibrous adhesions and fibrinous exudate. Both lungs were dark red, purple mottled and mostly firm. On section the surfaces were studded with purplish red circumscribed consolidated areas, 0.5 to 1.0 cm. in diameter.

Microscopically, the parenchyma contained numerous irregular and polypoid granulomas projecting into the lumens from the walls of alveoli or ducts. The granulomas were composed of spindle-shaped cells with their long axes parallel. The predominant cells had flat basophilic nuclei with central clumping of the chromatin. Other forms manifested polymorphous or oval basophilic nuclei and eosinophilic cytoplasm. Some cells were multinucleated. Polymorphonuclears, lymphocytes and red cells also were apparent. The stroma was loose and porous, and contained fine basophilic mucoid material. In some fields fibrin was discernible in the stroma. Elsewhere marked vascular congestion was present; pigmented and foamy phagocytes, hyaline membrane and edema were prominent. Occasional small foci of polymorphonuclears were observed. Cuboidal cells formed a covering over the granulomas, sometimes in several layers: some of these cells were phagocytic and contained occasional fat droplets. Many granulomas obstructed the alveolar orifices, and the adjoining alveoli were partially collapsed.

Case 8.—E. S., a white girl 16 years old, was admitted to the Children's Hospital with a complaint of abdominal pain and nausea of one day's duration. The past history revealed that she had had rheumatic fever at 9 years of age and had been in bed one and a half years at that time. Similar attacks had occurred at various times subsequently, the last, six weeks before admission. The temperature on admission was 102.0 F.; the pulse rate 132; the respiratory rate, 36. The lungs were clear and resonant. The heart was enlarged to the left nipple line, and a palpable systolic thrill and a loud systolic murmur were obtained over the apex. The murmur was transmitted to the left axilla and over the back. The abdomen was rigid and tender, especially in the lower left half.

On admission the white blood cell count was 19,800, with 77 per cent polymorphonuclears; the red cell count was 4,180,000. Later the leukocyte count rose to 26,500, with 79 per cent polymorphonuclears. A roentgenogram of the chest demonstrated mottling through the entire right lung and the upper lobe of the left. The heart was markedly enlarged.

The diagnosis of perforating appendicitis with formation of an abscess was made. However, the patient's condition was considered too poor for operative intervention. Treatment was undertaken with sulfadiazine, blood transfusions and Wangensteen drainage. Ten days after admission ileostomy was performed and a catheter inserted to relieve symptoms of intestinal obstruction. The peritoneal cavity contained much turbid fluid. The subsequent course was steadily downhill, and death occurred eight days after operation.

At a restricted postmortem examination the following diagnosis was made: Perforating appendicitis with periappendical abscess and diffuse peritonitis; rheumatic pancarditis (acute and chronic endocarditis of the mitral and aortic valves with mitral stenosis, myocarditis and pericarditis); cardiac hypertrophy; bilateral lobular pneumonia.

Gross examination made through diaphragmatic incisions revealed fibrous pleural adhesions superiorly on both sides and patchy nodules of consolidation in both lungs. No weights were obtained.

Microscopically, some alveoli contained scattered extravasations of red blood cells; others, "heart failure" phagocytes. Small granulomas were numerous in all parts of the lungs; many were subpleural in location. In some single low power fields as many as ten could be seen. The lesions varied in shape; many were polypoid, and some were oval or stellate. They were situated usually in the alveolar ducts, but some extended into the alveoli. The attachment of the granulomas to the alveolar or the duct wall was either slender or broad. The granulomas had a delicate, porous, in parts basophilic and mucoid stroma. They contained large numbers of wavy fine fibers, most of which yielded a positive collagen reaction with Van Gieson's stain. The lesions were scant in cells, and the latter tended toward polarity. Fibroblasts and lymphocytes were observed; occasionally, a cell with abundant eosinophilic cytoplasm and a polymorphous nucleus was discernible. Capillaries were present in a few granulomas. The outer surfaces of the latter were covered with cuboidal cells. No acute inflammatory changes were noted. The blood vessels and bronchi were normal.

COMMENT

Clinical Findings.—The clinical symptoms in our group of cases were indefinite. Cases like that of Nittono and Hoshiyama,[10] in which the picture of acutely developing lobar pneumonia appeared in a patient with rheumatic fever, are probably rare. Sometimes severe lesions of the lungs are found even in the absence of clinical signs (Debré, Marie, Bernard and Normand[13]). However, in our material rheumatic infection associated with specific pulmonary involvement gave sudden rise to the following syndrome: fever, cough, bloody sputum and elevation of the white blood cell count (usually over 15,000). Dyspnea was frequent and severe; Hadfield pointed out the significance of hyaline lining membrane in its pathogenesis; he emphasized the serious interference with gaseous exchange as a result of the sealing off of many alveoli by this membrane. Cyanosis and pain in the chest were less important than in pneumonia uncomplicated by heart disease. In some patients the pneumonia appeared late in the clinical course. The condition showed little or no response to sulfonamide compounds; some authors have claimed beneficial effects with salicylates. Bacteriologic findings were negative, and roentgen changes with one exception (case 4) were not of specific diagnostic value. In case 4 the widely disseminated fine stippling and mottling resembled early miliary tuberculosis. Such a roentgen finding in association with the symptoms mentioned would suggest the clinical diagnosis of rheumatic pneumonia. The stippling and mottling are probably the result of the focal necrosis which we shall soon describe.

13. Debré, R.; Marie, J.; Bernard, J., and Normand, E.: Presse méd. **45**:273, 1937.

PATHOLOGIC CHANGES

Gross.—The lungs in the cases reported here were heavier than normal and presented areas of lobular or confluent lobular consolidation with purplish red, sometimes granular congestion apparent on section. Fibrinous pleuritis was an associated finding in several instances. The gross changes were not of definite diagnostic value so far as a specific rheumatic pulmonary process is concerned. Other authors have stressed the rubbery consistency of certain areas in the lung as fairly characteristic of rheumatic pneumonia.[14] We have not been impressed by such alterations in elasticity and agree with Masson, Riopelle and Martin,[5] who stated "that the characteristic changes are not visible grossly."

Microscopic.—It should be stressed that the microscopic pictures of different areas and adjacent fields in the same lung present great variability. In some cases many blocks were examined before characteristic lesions were found.

(*a*) Fibrinous exudate: This was present in all our cases of active pulmonary inflammation. It was much more extensive and much denser than the fibrinous deposits seen in ordinary types of pneumonia. We observed it in the form of dense plugs within the lumens of the alveoli or ducts (fig. 1). In the alveolar ducts the fibrinous masses frequently blocked off the openings of the alveoli. Older inspissated fibrin of this character sometimes produced lining membranes.

(*b*) Focal fibrinoid necrosis with alveolitis: An important histologic feature, probably closely related to fibrinous exudation, was the fibrinoid necrosis in the alveolar wall itself. It consisted of homogeneous eosinophilic swelling of the wall associated with necrosis, which was manifested by numerous pyknotic and fragmented nuclei. These areas were infiltrated with polymorphonuclear leukocytes in a fairly characteristic manner. The polymorphonuclears were frequently concentrated in the alveolar walls and peripherally in the adjacent alveolar lumens, but the number decreased in the center. This distribution of the polymorphonuclears was different from that of ordinary types of pneumonia and gave the impression of a peppering or fine spraying (fig. 2). Elastic fibers could not be demonstrated in these foci.

(*c*) Vascular changes: An outstanding feature of rheumatic infection in general is the alteration in the walls of blood vessels. In the lungs in our material arteriolitis was observed repeatedly; it was manifested by fibrinoid swelling of the vessel wall, with infiltration by polymorphonuclears and lymphocytes. Frequently, the inflammatory changes extended into the perivascular tissues and offered a striking resemblance to periarteritis nodosa (fig. 3). In the case of a 24 year old woman, not included in this report, there was marked perivascular fibrosis, comparable to the perivascular myocardial scars seen in old rheumatic heart disease. Although recent pulmonary lesions were not demonstrable, rheumatic valvular heart disease was present, and we believe that these vascular changes might be the end result of rheumatic involvement.

(*d*) Other inflammatory features: Elsewhere in the parenchyma were focal and diffuse inflammatory infiltrates in varying degrees. The cells were chiefly mononuclear foamy phagocytes, less commonly other forms of leukocytes, with admixture of red cells. Many of the septal cells of the alveolar walls were swollen and proliferated, forming an incomplete lining (fig. 4). Except for case 2, the bronchi and bronchioles showed nothing remarkable.

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14. (*a*) Naish, A. E.: Lancet **2**:10, 1938. (*b*) Tragerman, L. J.: Arch. Path. **22**:566, 1936. (*c*) Gouley.[8] (*d*) Nittono and Hoshiyama.[10]

(*e*) Granulomas: These distinctive lesions in the course of rheumatic pulmonary inflammation have been observed by others. Masson, Riopelle and Martin [5] gave a most thorough description of them and called them *bourgeons conjonctifs* (connective tissue buds). In our paper we have designated them as granulomas. Since neither of these terms connotes any specificity for these characteristic lesions, they might be called Masson bodies appropriately.

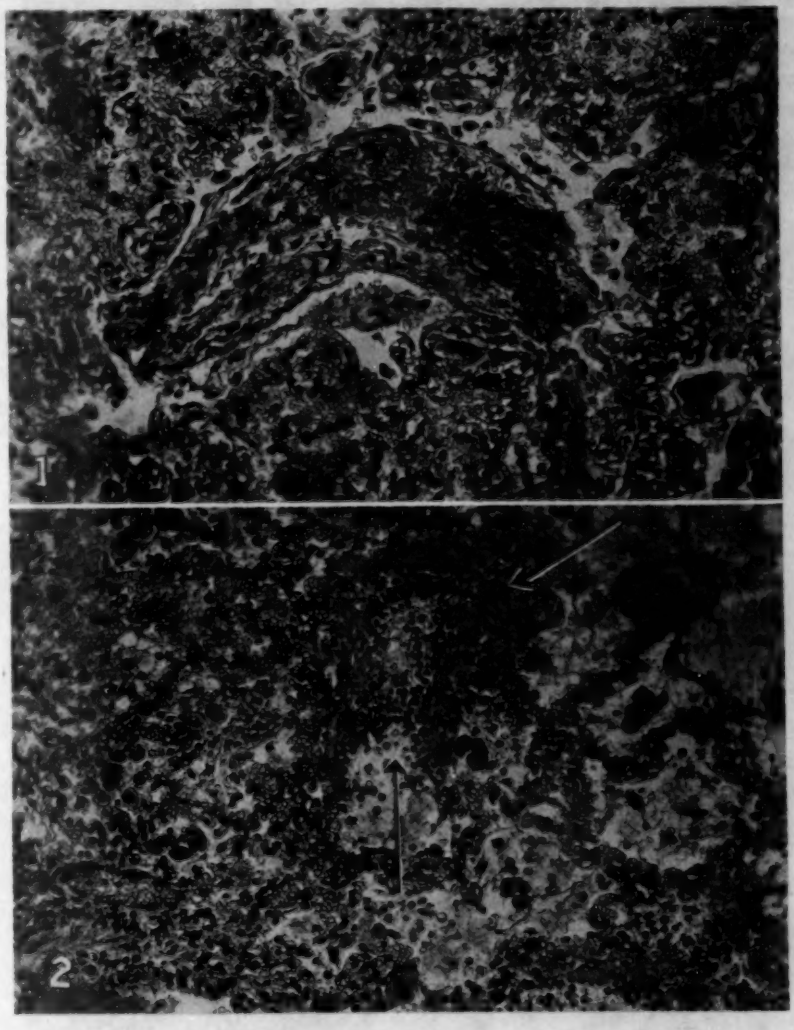

Fig. 1 (lung, case 1).—A dense fibrinous plug is apparent in an alveolar duct. Note numerous mononuclear cells in the exudate in the adjoining alveoli. × 160.

Fig. 2 (lung, case 1).—Fibrinoid necrosis is present in the alveolar wall as indicated. Pyknotic nuclei, nuclear fragments and small foci of polymorphonuclear infiltration are present also. Note the mononuclear phagocytes in the lumens of other alveoli. × 160.

On superficial examination the granulomas appeared to be located in the interstitial tissue. However, closer study with the aid of serial sections and the application of elastic fiber stains revealed that the lesions were situated mainly within the alveolar ducts, with frequent extension into the alveoli. Only rarely did small

independent intra-alveolar granulomas occur. Variable degrees of atelectasis were noted in alveoli whose orifices were thus occluded. In some low power fields there were as many as ten to fifteen granulomas (fig. 5). They appeared as round, oval or irregular structures with varying cellular concentrations; some resembled renal

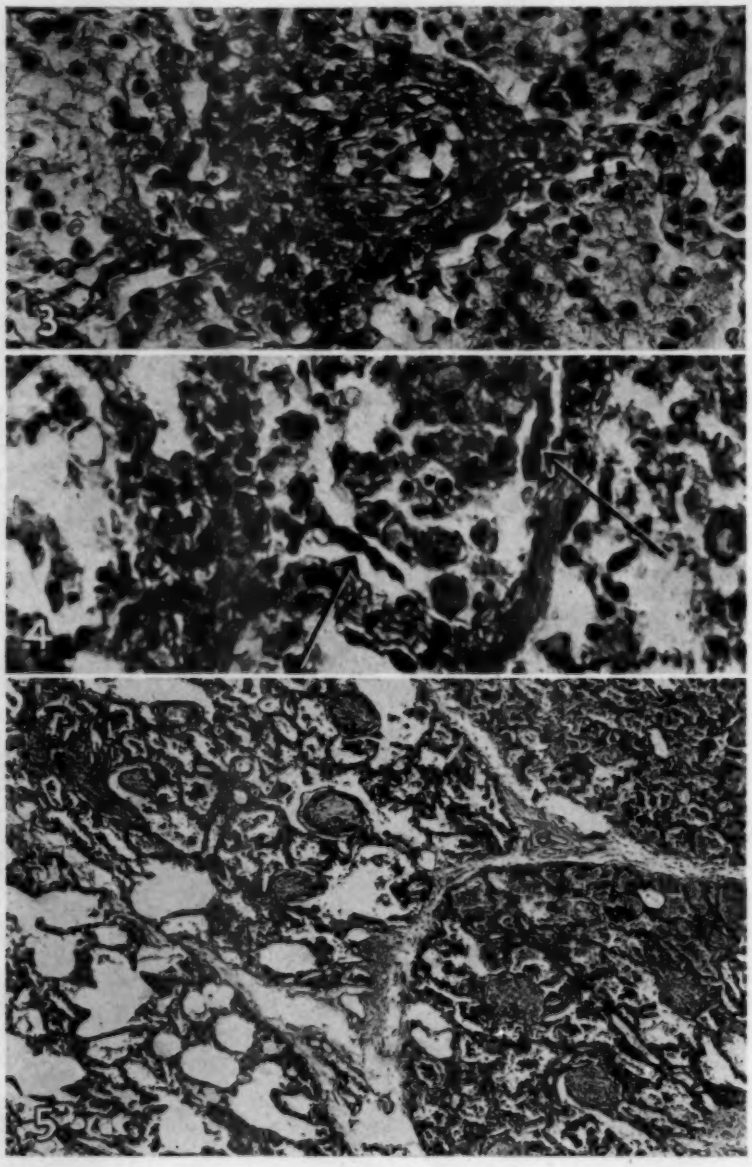

Fig. 3 (lung, case 2).—Arteriolitis is shown. The inflammatory reaction is acute, includes many polymorphonuclear leukocytes and closely resembles periarteritis nodosa. × 425.

Fig. 4 (lung, case 4).—Note septal cell proliferation and incomplete lining in rows of three to five cells as indicated. Numerous mononuclear phagocytes are apparent in the exudate. × 400.

Fig. 5 (lung, case 7).—Numerous granulomas (Masson bodies) are visible. × 40.

glomeruli. Cuboidal lining cells partially or completely covered many of the granulomas.

High power magnification of the granulomas observed revealed pleomorphic cellular elements. The central parts contained many cells with flat spindle-shaped basophilic nuclei and scanty neutrophilic cytoplasm; the cells showed a definite tendency toward polarity. Another prominent form had a round, oval or kidney-shaped nucleus, which was less intensely basophilic. The cytoplasm was abundant and well demarcated, and presented variable staining qualities: In some cells it appeared slightly eosinophilic, in others it was mildly basophilic, and in many it was neutrophilic. The cells showed phagocytic tendencies in the occasional presence of golden brown pigment in the cytoplasm. Sometimes lymphocytes were seen in the stroma. The different cellular forms varied in concentration but the spindle-shaped fibroblast predominated. The stroma was loose, fibrillary and collagenous; it contained fibrinous and, in some instances, basophilic mucoid material. The fibrin was mostly loose and threadlike but occasionally was arranged in dense strands along the margins of the granulomas. Some lesions were poor in cells; others were highly cellular with little ground substance. As a rule, the granulomas were scant in capillaries; many displayed no vessels.

Another interesting cell type, found as a covering for the "Masson body," was a flattened or cuboidal, sometimes stratified form with a round or oval basophilic nucleus and abundant indistinct cytoplasm of a less basophilic type. The cells and their arrangement as a covering were morphologically identical with the septal cell lining of the alveolar walls, mentioned earlier.

COMMENT

In summary, then, the important pathologic observations include: characteristic granulomas in the alveolar ducts and alveoli; focal fibrinoid necrosis with alveolitis; arteriolitis; septal cell proliferation. The granulomas in particular present features which have not been reported heretofore in other conditions affecting the lung. These lesions are identical with the *bourgeons conjonctifs* of Masson, Riopelle and Martin.[5] No thorough description has been given in the literature of the United States. Tragerman [14b] in a brief report mentioned submiliary nodules in rheumatic pneumonia but gave no histologic details. Typical Aschoff bodies in the lungs such as those reported by Fraser [15] and by Gouley [8] were not observed by us, but we consider the granulomas to be their equivalents.

Comparison of granulomas in the lungs (fig. 6) with Aschoff bodies in the myocardium of the same subject (fig. 7) reveals the following definite similarities. Both are well defined structures of approximately similar size. The cellular components show a marked tendency toward polarity; the stroma is loose, delicate and mucoid and contains varying amounts of fibrin, lymphocytes, polymorphonuclear leukocytes and fibroblasts. However, there are fundamental differences. The myocardial Aschoff bodies are located in the interstitial connective tissue, whereas the pulmonary granulomas are within ducts and in part are intra-alveolar. Typical Aschoff cells are not found in the pulmonary granuloma, which contains a different type of large cell, as described.

SPECIFICITY OF THE LESIONS

The differentiation between the "Masson body" and the granulation tissue of organizing pneumonia is obviously important. Grossly, the lung in organizing pneumonia reveals fibrous changes that are usually detectable on palpation and are

15. Fraser, A. D.: Lancet **1**:70, 1930.

restricted to one or more lobes. On the other hand, the characteristic changes in rheumatic pneumonia cannot be identified with certainty by gross examination. Microscopically, the granulation tissue in organizing pneumonia is usually diffuse; it fills and obliterates large numbers of adjoining alveoli and ducts. It may proliferate from one alveolus into another through the pores of Kohn and appears richly vascular. The "Masson body," in contrast, is discrete, frequently polypoid, and scant in vessels; it does not extend through the alveolar walls.

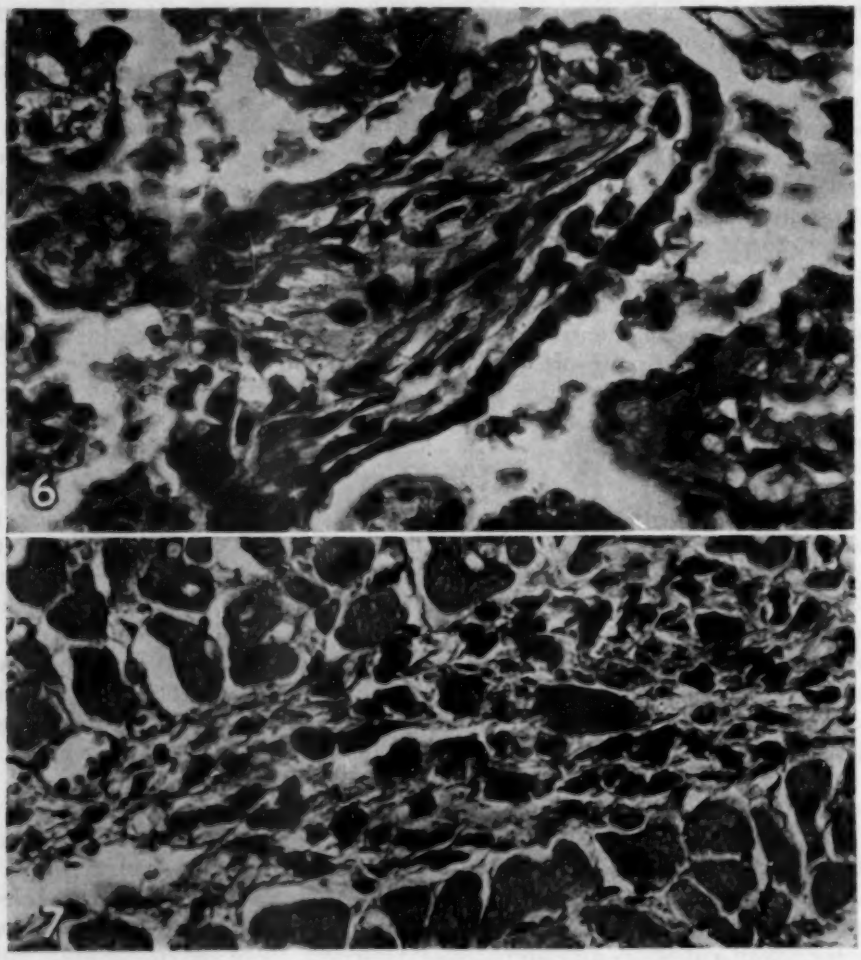

Fig. 6 (lung, case 7).—Granuloma (Masson body) with external cuboidal cell lining. × 500.

Fig. 7 (heart, case 7).—Aschoff body in myocardium. Compare with figure 6, showing a Masson body from the same case.

In uncomplicated chronic passive congestion of the lungs the vascular engorgement, the fibrous thickening of the alveolar walls and the large number of intra-alveolar "heart failure" cells produce a characteristic picture, which is in no way comparable to the changes that we have described. Hypostatic lobular pneumonia or bronchopneumonia in patients with chronic passive congestion showed no distinctive features in our control series.

From these considerations it would appear that the "Masson body" is a fairly specific granuloma. Others [16] have indicated that the Aschoff body may be observed sometimes in nonrheumatic conditions. Similarly, we discovered the "Masson body" in a few instances wherein no previous rheumatic history was elicited. One was the case of a 73 year old man who died of coronary sclerosis. The lungs were somewhat atelectatic as a result of hydrothorax but otherwise were not remarkable. Microscopically, no recent inflammatory changes were discernible, but there were occasional granulomas which could be considered older, fibrosed "Masson bodies." Despite a few exceptions of this type we believe that the "Masson body" is a fairly specific granuloma.

Another pertinent question is whether the other lesions, such as the focal fibrinoid necrosis with alveolitis and the arteriolitis are consistent with those found in rheumatic infections in general. Rheumatic inflammatory changes in joints and endocardium are identical in many respects; fibrinoid degeneration and swelling (*Verquellung* of German authors) and arteriolitis, sometimes resembling periarteritis nodosa, are well known features. Since connective tissue is the site of specific rheumatic inflammation, there appears to be no reason to consider the lung framework immune. Rheumatic changes in the larger pulmonary arteries, as reported by Chiari,[1] by Von Glahn and Pappenheimer [17] and by Brenner,[18] were not impressive in our material. Mononuclear cell exudate and septal cell proliferation as seen in our cases were not in themselves of diagnostic value. However, these features are of interest from the standpoint of etiology, which we shall mention later. The genesis of these lesions has been repeatedly discussed by other authors and will therefore not be considered.

GENESIS OF THE "MASSON BODY"

The "Masson body" can be studied accurately only by means of serial sections. In our material many granulomas appeared to originate as papillary protrusions from the walls of the alveolar ducts or alveoli. In some instances a thin stalk of tissue connected the granuloma with the alveolar or the duct wall; in others the stalk had a broader attachment. Other granulomas appeared to be free in the lumens, although in serial sections they revealed attachment at some points. In sections made at 6 micron intervals the individual granulomas revealed surprising variation from one level to another. There was no tendency toward localization in any particular part of the lung. In this respect our findings differed from those of Masson, Riopelle and Martin,[5] who considered that the central portions of the lung were more commonly affected. Some granulomas seemed to have originated by organization of fibrinous plugs; this was emphasized by Masson and his associates [5] and by Hadfield.[7] In others, however, it was possible that the granuloma had grown outward from the alveolar or the duct wall without associated fibrinous exudation.

Both possibilities conform with some of the present views on the genesis of rheumatic granulomas in general: Both Fahr [2] and Pagel [19] held that the lesions could arise independent of preliminary fibrinoid degeneration. This is in contrast with the opinion of Klinge,[4] who thought that the "early infiltrate" invariably

16. Kalbfleisch, H. H.: Verhandl. d. deutsch. path. Gesellsch. **30**:73, 1937. Clawson, B. J.: Arch. Path. **8**:664, 1929.
17. Von Glahn, W. C., and Pappenheimer, A. M.: Am. J. Path. **2**:235, 1926.
18. Brenner, C.: Arch. Int. Med. **56**:1189, 1935.
19. Pagel, W., in Kallós, P.: Fortschritte der Allergielehre, Basel, S. Karger, 1939.

preceded the granulomatous formation. He expressed the belief that fibrinoid masses were deposited between the connective tissue fibrils as a preliminary stage.

The ages of the granulomas can be estimated to some extent. In those that originated from fibrinous plugs, organization by young fibroblasts was sometimes observed. Older granulomas showed more or less complete absence of fibrin; basophilic mucoid material became more prominent in the stroma. At an even later stage, collagenous fibers, some swollen and coarse, appeared; the cellularity decreased, and the fibroblasts tended to predominate. The oldest lesions were fibrous and relatively acellular.

By reference to the clinical histories it was possible to establish the fact that the "Masson body," like the Aschoff body in the heart, may remain as a characteristic structure for an indefinite period. In our series, however, we were unable to determine the time required for the development of the "Masson body" before fibrous involution occurred. In case 7 the lesions could not have been more than three months old. In case 5 the last rheumatic attack occurred five years before death and the granulomas were still apparent. Thus the "Masson body" may persist for years after the other inflammatory changes found in rheumatic lungs have disappeared.

The origin of the cells which formed a covering for the "Masson body" is of interest. We believe that they were derived from alveolar septal cells. Morphologically, these forms were identical with the alveolar cuboidal lining cells, which proliferate readily in various inflammatory conditions (Geever, Neubuerger and Davis [20]). In many instances the two cell forms were found in the same field and could be compared easily. In the granulomas that resembled renal glomeruli the continuous transition from "parietal" to "visceral" septal cells could be traced. The cuboidal covering cells were quite independent of fibroblasts and other cells in the substance of the "Masson body."

ETIOLOGY

Masson, Riopelle and Martin [5] expressed the opinion that the rheumatic involvement of the lungs that they described was related possibly to environmental conditions peculiar to Montreal, Canada; the complete pathologic picture, as observed by them, had never been reported before. Our studies indicate that the same type of rheumatic pulmonary change occurs elsewhere. It is of interest in this regard that Colorado has a high incidence of rheumatic fever, approaching that of the New England states. This is contradictory to the opinion that high altitude and dry, sunny climate, which prevail in Colorado, protect against rheumatic infection.

This morphologic study has not furnished additional information on other etiologic problems of rheumatic fever. Bacterial stains revealed no organisms in most fields; only a few clumps of diplococci or streptococci were found. These organisms did not impress us as etiologic agents. With regard to allergy, it may be of interest that in some of our cases arteriolitis greatly resembling periarteritis nodosa was prominent. Friedberg and Gross [21] called attention to a possible relation between this condition and rheumatic fever. Recently, Rich [22] claimed a direct etiologic connection between allergy and periarteritis nodosa.

Some histologic features in our series were suggestive of virus as a cause. These included the presence of large numbers of mononuclear cells in the exudate,

20. Geever, E. F.; Neubuerger, K. T., and Davis, C. L.: Am. J. Path. **19**:913, 1943.
21. Friedberg, C. K., and Gross, L.: Arch. Int. Med. **54**:170, 1934.
22. Rich, A. R.: Bull. Johns Hopkins Hosp. **71**:375, 1942.

stimulation of septal cells with lining of alveoli with septal cells and vascular damage. We referred to these features in a previous report on pneumonia associated with chickenpox.[23]

SUMMARY

In a series of 63 cases of active and quiescent rheumatic fever there were 8 with pulmonary inflammation showing distinctive features. These features were: peculiar granulomas in the alveolar ducts and alveoli; focal alveolitis with necrosis, fibrinous exudation and hyaline lining membranes; arteriolitis; mononuclear cell exudation and septal cell proliferation.

The term "Masson body" is suggested for the rheumatic pulmonary granuloma, which is considered to be an equivalent of the Aschoff body in the heart.

23. Waring, J. J.; Neubuerger, K. T., and Geever, E. F.: Arch. Int. Med. **69**:384, 1942

CHOLESTEROL LYSIS IN ATHEROMA

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In a previous publication [1] the following sequence was demonstrated in the rabbit fed cholesterol:

A. The esterification of ingested cholesterol in the liver.

B. The deposit of esters in liver cells in massive amounts.

C. The phagocytosis of ester cholesterol as particulate matter by the Kupffer cells, which thus became foam or lipoid cells.

D. The freeing of the Kupffer cells from the walls of the liver sinusoids and their delivery into the blood stream or their invasion of lymphatics.

E. The passage of the foam cells through the right side of the heart and the lungs.

F. The invasion by these cells of the subendothelial layer of the aortic (arterial) intima and the initiation of atherosclerotic lesions.

This study included observations that when lymphatics were obstructed by massed foam cells the cells after long stagnation split the esters in their contents and brought the cholesterol into solution in fat which stained purplish blue with nile blue sulfate. The inference is that under these conditions the ameboid foam cells secrete or absorb an excess of fatty acids capable of holding the cholesterol in solution. Hirsch [2] disclosed that human fat normally becomes saturated with 4 per cent of cholesterol but can dissolve up to 13 per cent after the addition of fatty acids. Solution of the cholesterol in the foam cells is associated with a loss of anisotropism.

It was also found [1] that feeding of cholesterol over long periods would produce in the experimental rabbit cirrhosis of the liver, enlargement of the spleen and chronic nephritis, a triad of lesions thought by Gye and Purdy [3] to be characteristic of chronic intravenous silica poisoning. Excess cholesterol is therefore an irritant comparable to but milder than silica.

Two quite distinct conditions may arise in the human body as the result of the presence of excess cholesterol esters in the arterial intima. The first of these, atheroma or atherosis (Aschoff), is a temporary process, the cholesterol being removed from the intima before it can cause lasting damage to the vessel wall. Atherosclerosis, on the other hand, is a permanent chronic process due to the prolonged stay of the cholesterol esters in the intima. It is responsible for most of the important lesions listed under arteriosclerosis.

In atheroma one is dealing with a reversible process. Cholesterol esters are carried into the subendothelial layer of the intima by Kupffer cells and are then removed by the procedure to be described. Atheroma is met with particularly in youth. Yellow streaks or spots in the aorta may be found in infancy when the metabolism of cholesterol is not adequate to cope with the quantity of the substance

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From the Medical Examiner Service, Suffolk County, Mallory Institute of Pathology.

1. Leary, T.: Arch. Path. **32**:507, 1941.
2. Hirsch, E. F.: Arch. Path. **25**:34, 1938.
3. Gye, W. E., and Purdy, W. J.: Brit. J. Exper. Path. **5**:238, 1924.

in an exclusive milk diet. Puberty is a period in which cholesterol metabolism is perhaps rendered unequal to the task of establishing sterol balance as the sex hormones become active. Independently of these periods situations may arise that lead to cholesterol overflow at any time in life.

In studies of atheroma [4] it was established that the reversible character of the lesions was dependent on the removal of the excess cholesterol from foam cells by fixed cells and the apparent solution of the cholesterol in a fatty material staining with sudan IV within the cells. In paraffin sections stained by Mallory's phosphotungstic acid–hematoxylin and aniline blue stains the fixed cells were seen to be producers of fibroglia fibrils and were therefore fibroblasts. The metamorphosis of the cholesterol was accompanied by loss of anisotropism of the fat drops in the fibroblasts.

With progressing age the body gradually loses the power to remove excess cholesterol from the arteries. The lipolytic fibroblasts continue to function for some time but with little effect on advanced lesions. The ascending aorta, however, retains the power to remove excess cholesterol even into old age. This part of the vessel hangs free with no anchorage by branches, and there are no breaks in the continuity of the media as it encircles the vessel. Whether these anatomic peculiarities are factors in preventing the development of atherosclerosis or not, advanced lesions of atherosclerosis are less common in the ascending aorta above the ring than in other parts of the vessel.

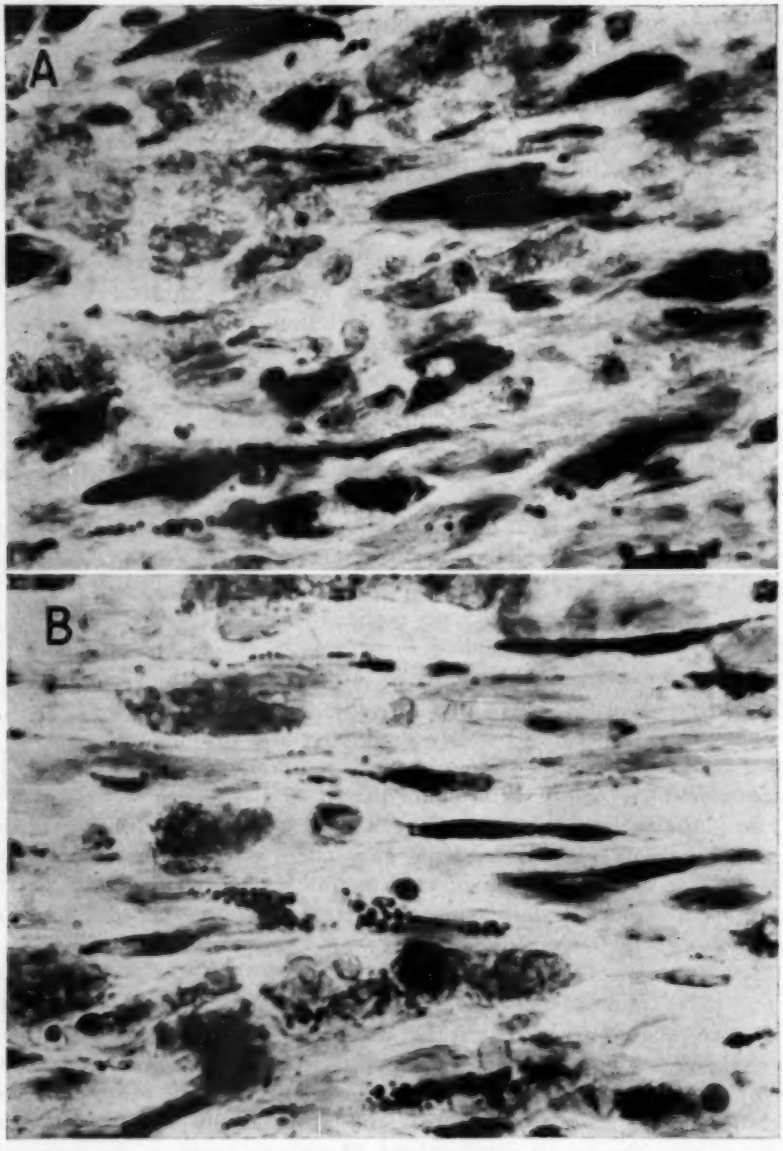
As is true in the cholesterol-fed rabbit, the ascending aorta in man is a favorite site for invasion by cholesterol-laden phagocytes. The lipoid cells gradually accumulate in given centers until compact masses, large enough to be seen grossly, are produced. These lesions become pinhead size or larger. They appear to occur in crops, perhaps corresponding to periods of excess cholesterol in the diet. The surface of the vessel may be actually beset with collections of discrete lesions of essentially the same size, color and probable age. The fresh lesions tend to be bright orange. As time goes by they gradually fade through yellow, pale yellow and gray. This succession of changes can be followed in a series of aortas and is so constant that there can be little doubt that it is a sequence. In frozen sections of the lesions the earliest (orange) stage is richest in cholesterol, an intermediate (yellow) stage shows less fat, and the gray stage is free from material staining with sudan IV. The mechanism of cholesterol removal is evidently activated by the irritant action of cholesterol since nodular lesions of massed lipoid cells are permitted to develop to considerable size and age before removal of cholesterol begins.

In sections of pinhead lesions in which the defense mechanism is active the fibroblasts are distinguished from the ameboid foam cells by their elongated processes, their production of fibroglia fibrils and their content of fat-staining material in drops of unequal but relatively large size. In the foam cells the droplets of cholesterol esters are very small and of strikingly equal size. A further distinction is that the fat in the lipolytic fibroblasts becomes isotropic, showing at most in early stages rare crystals and in late stages none. The cholesterol esters in the foam cells are anisotropic and apparently remain so until the cells are robbed of their lipoid.

The illustration shows frozen sections of pinhead lesions of the ascending aorta stained with nile blue sulfate. In general the cells occur in bands running transversely and paralleling the surface of the intima. Fibroblastic lipolytic cells lie

4. Leary, T.: Arch. Path. **21**:419, 1936.

between the bands of foam cells. The deep, more solid color (purplish red) of the fibroblasts is in contrast to the paler granular tone (yellow or pink to light red) of the foam cells. The fibroblasts tend to be more discrete and sharply out-



A, deep-staining lipolytic fibroblasts lying among groups of lipoid (foam) cells. Most of the lipoid content of the foam cells has been transferred to the fibroblasts. Apparently free deep-staining drops are within cell processes (rows) or have been set free by rupture of the cells in sectioning. Frozen section; × 1,500.

B, microscopic field neighboring that shown in *A*. In the midfield is a lipolytic cell and below a portion of another in which deep-staining droplets of varying size are seen. The color and texture of the ovoid lipoid cells between these lipolytic cells bring out clearly the contrasting character of the two cell types. The nucleus of the upper lipolytic cell referred to lay in the unstained center of the cell but at a lower plane. Frozen section; × 1,500.

lined, while the foam cells occur in masses. In the figure *A* illustrates the color contrast between the two types of cells, while *B* shows in its central region a cell which happened to lie close to the cut surface of the frozen section and which exhibits the deep-staining fat drops in the unstained cytoplasm. The droplets in the foam cells are too small to be distinguished as such, as is usual in frozen sections. Here and there in both *A* and *B* occur drops of deep-staining material in cell processes or free. Most of the lipolytic cells are so filled with drops of deep-staining fat that they appear to be solid structures.

The process of removal of excess cholesterol from arterial deposits appears, then, to be due to the functioning of fibroblasts that come to contain, by secretion or perhaps by absorption, an excess of fatty acids in which the cholesterol is dissolved. The splitting of the cholesterol esters, a comparatively simple process, is probably also due to their activities. The treatment of the cholesterol does not stop with the abstraction of the substance from foam cells and its solution in fluid fat in lipolytic fibroblasts. The lipoid then disappears from the affected area. The lipolytic cells are fixed cells and cannot transport material. There is no evidence locally that other cells are active in the disposal of the substance. The disappearance of the lipoid from the lesions is apparently due to further metabolism within the bodies of the fibroblasts, which return to their normal shape and appearance after the fat vanishes. The foam cells stripped of their ester content tend to disintegrate and disappear from the lesions. As the cholesterol is removed, the loose-textured fibroblastic tissue of the gray stage is gradually flattened out, little or no collagen is produced, and finally the lesions can no longer be seen grossly. There remain locally slight thickenings of the intima requiring microscopic examination for their detection.

The readiness of the ascending aorta to succumb to atherosclerosis in the absence of the defense mechanism is illustrated in syphilitic aortitis.[5] In that condition the overwhelming changes created in the intima by the productive growth of connective tissue about the vasa vasorum, as these vessels erupt through the media, apparently abolishes the defense. Lipoid cells invade the aortic wall as the syphilitic process wanes, not only from the intimal surface but also through the vasa vasorum. The result is a continuous atherosclerotic process of the highest grade from the aortic ring to the lower margin of the syphilitic lesions, usually in the thoracic aorta.

SUMMARY

A defense mechanism removes excess cholesterol from the arteries of youth and from the ascending aorta even in advanced age. The cholesterol is transferred from wandering lipoid foam cells to fixed fibroblasts in which the cholesterol esters are split, anisotropism is lost, and the cholesterol is brought into solution in an excess of fatty acids; solution is followed by its disappearance from the lesions. The reversible character of the lesions in atheroma furnishes additional evidence that excess cholesterol is the cause of atherosclerosis. As the cholesterol is removed from the lesions, they stop progressing toward atherosclerosis and subside.

The importance of the defense mechanism demonstrated is that it is the only known agency for the elimination of excess cholesterol from tissues. It is also the first specific example of cholesterol metabolism so far presented. Furthermore, studies of nature's method of removing excess cholesterol from lesions may suggest a new approach to the treatment of atherosclerosis.

5. Leary, T.: New England J. Med. **223**:789, 1940.

EOSINOPHILIA OF THE SPLEEN ASSOCIATED WITH SUDDEN DEATH

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In reviewing the histologic sections of routine autopsies striking numbers of eosinophilic leukocytes were found in the spleen in several instances. Inasmuch as the obvious possible causes for this eosinophilia, such as dermatitis or allergic conditions, were absent, a common denominator was sought. In the first few cases, at least, this common factor appeared to be fatal trauma. Accordingly, the immediate problem resolved itself into collecting a sufficient number of traumatic and suitable control cases in order to determine whether or not there was a statistically significant difference in the incidence of splenic eosinophilia in these groups.

MATERIAL AND METHODS

Hematoxylin and eosin-stained sections of spleen from the following groups of cases were examined:

1. Cases in which death was due to trauma from a ground vehicular or an airplane accident, a gunshot wound, a fall, a blunt force or an explosion—138 cases:
   - (*a*) 100 cases in which sudden death occurred immediately after the trauma.
   - (*b*) 19 cases in which death occurred in a period from approximately ten minutes to ten hours after the trauma.
   - (*c*) 19 cases in which death occurred in a period from ten hours to ten days after the accident.

2. General controls—100 cases: The causes of death in this group were those one might expect in a general hospital, including such conditions as meningitis, neoplasms, bacterial endocarditis, nephritis, cardiac failure, pneumonia and postoperative complications.

3. Special controls—131 cases:
   - (*a*) 100 cases in which sudden death was due to a nontraumatic cause, such as coronary occlusion or pulmonary embolism.
   - (*b*) 31 cases in which death occurred relatively rapidly, in a period of several minutes, from carbon monoxide poisoning, intracranial hemorrhage, massive hemorrhage as from a severed artery, etc.

The patients were men ranging in age from 19 to 75. Ten per cent of them were Negroes. Autopsies were performed one to twenty-seven hours after death.

The presence of eosinophilic leukocytes in the spleen was graded on the basis of 1 to 4 plus, each plus representing approximately one eosinophilic leukocyte per high power field. Spleens in which 2 or more eosinophilic leukocytes per high power field were found were arbitrarily regarded as showing eosinophilia. It was necessary to discard an occasional case because the sections were stained either so intensely or so faintly with eosin as to make accurate differentiation of cells questionable. Only those cells with unequivocal, refractile, relatively large eosinophilic granules were included, and care was taken to distinguish them from the amphophilic or pseudoeosinophilic leukocytes.

FINDINGS

The incidence of splenic eosinophilia (2 or more eosinophilic leukocytes per high power field) in cases of death occurring immediately or within a few hours after an accident as contrasted with the incidence in general controls was as follows:

Group	Cases	Number with Eosinophilia of Spleen			Total with Splenic Eosinophilia	Percentage with Splenic Eosinophilia
		++	+++	++++		
1. Immediate traumatic death	100	19	33	29	81	81
2. Death due to such conditions as meningitis, neoplasm, nephritis, etc. (routine controls)	100	6	2	1	9	9

From the Army Medical Museum, Institute of Pathology, Washington, D. C.

These data indicate a strikingly significant difference in incidence of splenic eosinophilia; in 81 per cent of the traumatic cases this condition was demonstrated, opposed to only 9 per cent of the controls. Not only was the incidence of cases with splenic eosinophilia remarkably greater in the traumatic group, but the spleens in over three fourths of this group showed eosinophilic leukocytes to the extent of 3 or 4 per high power field, whereas merely one third of the controls with splenic eosinophilia presented such numbers. Indeed some of the traumatic cases with grades of 4 plus showed as many as 20 eosinophils per high power field. Furthermore, included in the 9 controls with splenic eosinophilia were cases of Hodgkin's disease, exfoliative dermatitis and meningitis in which serum was administered twenty-four hours before death. The eosinophilia in these 3 cases is not surprising, and such instances might have been eliminated from a control series.

In order to determine whether or not such splenic eosinophilia persists, the spleens of patients who survived accidents for various periods were examined, with results as follows:

Survival Period After Trauma	Cases	Number with Eosinophilia of Spleen	Percentage with Eosinophilia
Essentially no survival	100	81	81
10 minutes to 10 hours	19	8	42
10 hours to 10 days	19	3	16

It seems clear from this tabulation that splenic eosinophilia occurs with greater frequency in those persons who succumb practically instantly to trauma than in those who survive the accident for even a few hours. Similarly, the probability of the occurrence of splenic eosinophilia appears to be diminished with increasing length of survival.

In order to determine whether or not trauma is in fact the agent responsible for the increased numbers of eosinophilic leukocytes in the spleen, a variety of other cases were studied in which death occurred suddenly or at least relatively rapidly over a period of minutes. Of the material available, spleens from ambulatory patients who had suffered coronary occlusion and immediate death seemed to constitute a logical control. The instances of sudden death due to coronary occlusion were separated into two groups on the basis of the presence or the absence of myocardial infarcts. This was done on the premise that if organic evidence of an infarct was found the practically symptomless myocardial lesion had been present at least several hours before death. These two groups of patients who died suddenly were then compared with a third group who survived coronary occlusion from one hour to ten days. The results were as follows:

1. Of 32 cases of sudden death due to coronary occlusion without evidence of myocardial infarction, there was eosinophilia of the spleen in 30 (94 per cent).
2. Of 18 cases of sudden death due to coronary occlusion with gross or microscopic evidence of myocardial infarction, there was eosinophilia of the spleen in only 6 (33 per cent).
3. Of 30 patients who survived coronary occlusion from one hour to ten days, there was eosinophilia of the spleen in only 8 (27 per cent).

The following additional miscellaneous cases of relatively rapid death were studied:

Type of Case	Cases	Number with Eosinophilia of Spleen
Pulmonary embolism	20	7
Carbon monoxide poisoning	15	8
Exsanguination	7	6
Intracranial hemorrhage	5	4
Anesthetic death	4	2
Total	51	27

The evaluation of this group is obviously limited by the heterogeneity of the types of cases as well as the small number of cases. However, although the percentage of cases in which there was eosinophilia of the spleen is appreciably below that in the groups in which sudden death was due to trauma and that in the groups in which sudden death was due to coronary occlusion (without infarct), it nevertheless is significantly above that observed in the routine, unselected controls.

COMMENT

This initial study indicates a remarkably selective occurrence of great numbers of eosinophilic leukocytes in the spleens of persons who die instantly following trauma. A hint of such a relationship was offered in the observation by several investigators that eosinophilia of the spleen was "not infrequent" in cases of sudden violent death. Of equal interest is the occurrence of an even more striking incidence of splenic eosinophilia in a group of cases in which there has been no factor of trauma; namely, cases of sudden death from acute coronary occlusion in which there has been no time for the development of a myocardial infarct. In both these groups the incidence of splenic eosinophilia appears to diminish with protraction of the survival period. There is still a third miscellaneous group of cases of nonviolent, relatively rapid death in which the incidence of splenic eosinophilia is suggestively greater than in the group of general controls. This third group merits considerable expansion before unequivocal conclusions are drawn.

With these few facts, one is at a loss to account for the mechanism of the mobilization of eosinophilic leukocytes in the spleens of persons suffering practically instant death from violence or from coronary occlusion. It remains to be determined whether or not there is postmortem migration of these cells from other depots or possibly a postmortem transformation of other splenic cells into eosinophilic leukocytes. Or, indeed, is it possible that the cell content of the truly normal spleen is still unknown? After all, the studies in the literature are not based on normal spleens removed from normal living patients. As a remote approach to this question, the sections of 10 spleens removed surgically in treatment for various diseases were studied as part of the current investigation. Four of the 10 spleens showed mild eosinophilia. Each of the 4 patients had a blood dyscrasia and anemia which possibly provoked splenic eosinophilia. In other words, this limited study of 10 surgically removed spleens does not indicate a normal high content of eosinophilic leukocytes. The explanation of the selective splenic eosinophilia must await at least complete data on the eosinophil content of peripheral blood, of marrow and of the remainder of the organs of people dying suddenly. It is interesting that the spleens of reptiles, amphibians and certain birds are rich in eosinophilic leukocytes.[1] Moreover, in several instances of intense splenic eosinophilia, the cells were observed also in lymph nodes, the thymus gland and venous clots, so that it seems likely that such eosinophilia is excessive and not normally present. The analysis of the clues offered by comparative pathology might prove fruitful. Finally, in view of the probable relationship of histamine[2] and acetylcholine[3] to eosinophilia in the peripheral blood, one naturally wonders if the release of some such substance may not be concerned with the phenomenon.

1. Klemperer, P.: The Spleen, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 3, sect. 21, p. 1649.
2. Code, C. F.: J. Physiol. **90**:485, 1937.
3. Granzner, O.: Folia haemat. **63**:217, 1939.

SUMMARY

Conspicuous numbers of eosinophilic leukocytes were found in 94 per cent of spleens from patients dying immediately after coronary occlusion in whose hearts infarcts had not yet developed and in 81 per cent of spleens from persons succumbing instantly to violence. Only 9 per cent of routine, unselected controls were found to have eosinophilia as herein defined.

There is a distinct tendency for the eosinophilic leukocytes to disappear from the spleen as the period of survival following the accident increases.

The explanation of the phenomenon is not known.

CANCEROUS MIXED TUMOR OF THE URINARY BLADDER

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A small number of cancerous growths of the urinary bladder containing mesoblastic tissues with the characteristics of skeletal muscle or cartilage, some with epithelium, have been reported as mixed, carcinosarcomatous, embryonal and teratomatous tumors. The tissue composition of these tumors is similar to that of other mixed tumors of the genitourinary tract but apparently they comprise only a small fraction of all tumors of the urinary bladder. Among the many tumors of the urinary bladder excised or demonstrated at autopsies during the past twenty-five years and examined for histologic structure in the laboratory at St. Luke's Hospital, Chicago, only the one herein described was recognized as having the composition of these mixed tumors.

Published statements support the opinion that such growths occur rarely in the urinary bladder. Wilms's [1] monograph referred to 3 mixed tumors of the urinary bladder reported by Albarran, 2 of them with cartilage and 1 with striated muscle, and to the reports by Ordonez (1856), Shattock (1887) and Livio (1887). Hückel [2] stated that mixed tumors of the urinary bladder are rare, are usually polypoid, appear in the region of the trigone or the ureteral orifice, contain various mesenchymal tissues and occur predominantly in males. He listed a papillary tumor in the right ureteral orifice of a man aged 55 years which contained cartilage, smooth muscle and spindle and round cell sarcoma, reported by Shattock [3]; a tumor diagnosed as sarcoma in a man aged 72 years which was located near the left ureteral orifice and contained calcified osteoid tissues, cartilage and striated muscle described by Beneke [4]; a tumor diagnosed as chondromyxosarcoma, reported by Ried [5]; a pedunculated mixed tumor of the trigone attached below the left ureteral orifice of a boy aged 1½ years and containing islets of cartilage, bundles of smooth muscle but no epithelium, described by Hüsler,[6] and a pedunculated tumor of the trigone in a woman aged 23 years which was composed predominantly of smooth and cross-striated muscle tissues, recorded by Mönckeberg.[7] Pollack [8] described an infiltrative cancerous teratoma of the neck of the urinary bladder in a man aged 72 years. The epithelium was in masses and acini, some of these resembling embryonal renal glomeruli, and the stroma, like embryonal mesenchyma, contained islets of undifferentiated cartilage. Metastases in the lungs had a similar histologic structure. Fibers with cross striations were not found. Droschl [9] recorded a pedunculated tumor classified as osteoid sarcoma of the urinary bladder which was attached to the trigone medial to the right ureter in a man aged 67 years. It contained spindle cells resembling fibroblasts, large polymorphous cells, multinucleated giant cells and trabeculae of osteoid tissues and bone. Arnold [10] described a tumor as teratoid carcinosarcoma of the urinary bladder which was a pedunculated growth attached above the left ureteral orifice in a woman aged 43 years and composed of epithelial and mesenchymal tissues. The surface had a simple columnar and stratified epithelium. The deeper portions had gland structures, solid masses of cuboidal, cylindric or polygonal cells and aggregates of pavement cells with hornification. Mesenchymal tissues comprising the bulk of the tumor consisted of fibrillar connective tissues, spindle-shaped cells, bizarre multinucleated giant cells and masses of atypical cartilage. Of 8 other cases of carcinosarcoma reviewed in Arnold's report, only that reported by Pollack was a case of carcinosarcoma containing cartilage. A tumor of the right ureter described by Renner [11] in a

From the Henry Baird Favill Laboratory, St. Luke's Hospital.

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1. Wilms, M.: Die Mischgeschwülste, Leipzig, Arthur Georgi, 1899.

2. Hückel, R., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1934, vol. 6, pt. 2, p. 668.

3. Shattock, S. G.: Tr. Path. Soc. London **38**:183, 1886-1887.

4. Beneke, R.: Virchows Arch. f. path. Anat. **161**: 70, 1900.

5. Ried, K.: Ein Fall von Chondromyxosarkom der Harnblase, Inaug. Dissert., Erlangen, E. T. Jacob, 1913.

6. Hüsler, G.: Jahrb. f. Kinderh. **62**:133, 1905.

7. Monckeberg, J. G.: Virchows Arch. f. path. Anat. **187**:471, 1907.

8. Pollack, A. D.: Am. J. Path. **12**:561, 1936.

9. Droschl, H.: Deutsche Ztschr. f. Chir. **249**:785, 1938.

10. Arnold, W.: Zentralbl. f. allg. Path. u. path. Anat. **77**:52, 1941.

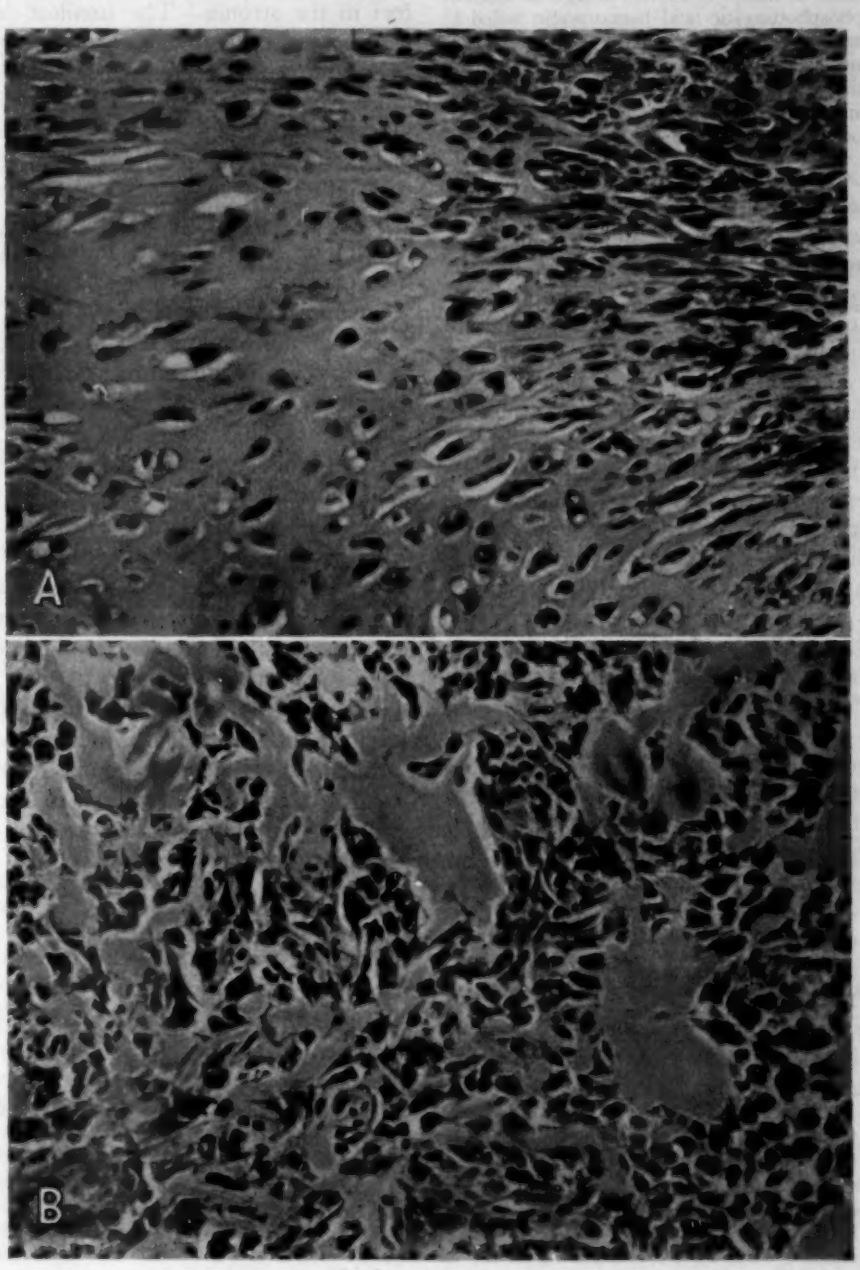
11. Renner, M. J.: Surg., Gynec. & Obst. **51**:793, 1931.

man aged 71 years and classified as carcinosarcoma had a structure similar to that of the tumor of the bladder recorded by Pollack.

REPORT OF A CASE

A white man aged 83 years entered St. Luke's Hospital in the care of Dr. T. P. Grauer May 27, 1943 because of hematuria which had been present for a month. Fifteen years before, the urinary bladder had been irrigated for hematuria, and at that time diabetes mellitus was recognized. There was no recurrence of urinary symptoms until his present complaint was noted.

On June 4, 1943, through a suprapubic incision, a tumor 3 to 5 cm. in diameter attached by a small pedicle on the left side of the bladder near the trigone was removed. The base of the pedicle was fulgurated and the bladder closed. Five days after the operation the patient died with symptoms of myocardial damage and hypostatic pneumonia. Permission for a postmortem examination was refused.



Photomicrographs illustrating significant portions of the mixed tumor of the urinary bladder (× 198): *A*, cartilage tissues merging with spindle and polymorphous cell sarcoma. *B*, hyaline bands with slight calcification and interstices containing angulated cells, including small myeloid cell forms.

The coarsely lobulated globular mass removed from the urinary bladder measured 5.5 by 4.5 by 3 cm. and weighed 35 Gm. The short pedicle was 2.5 cm. in diameter. At least two thirds of the mass was necrotic, red-brown and leathery. The other portions were gray.

The microscopic structure of the tissues was remarkable because of the mixed mesoblastic tissue content (figure). Islets of cartilage were scattered in a hyaline and fibrillar stroma along with elongated large spindle and polymorphous cells. Among the latter cells were many in mitosis. The more cellular tissues had spindle cells, large angulated cells and giant cells of the myeloid type. Portions of the dense hyaline trabeculae contained deposits of lime. A careful search in sections stained with phosphotungstic acid–hematoxylin failed to demonstrate tumor cells with striations characteristic of skeletal muscle. The epithelial components were limited to a thin surface layer of squamous epithelium and to small masses of similar cells nearby that seemed to be extensions into crevices.

These mixed tumors of the urinary bladder doubtless belong with tumors of comparable structure found elsewhere in the male and the female genitourinary tract in the region of the primitive wolffian duct. They contain mesoblastic tissues; some of them, also epithelium. On the basis of tissue structure and invasive qualities they are cancerous. Although most of these growths have occurred in the male urinary bladder, several have been observed in the female bladder. The criterion that cartilage or bone must be present in the tumor for the diagnosis of mixed tumor may seem arbitrary unless other considerations, such as position or gross features, establish beyond doubt the mixed tumor character of the growth. This is true especially for tumors in which only muscle fibers, either smooth or striated, are demonstrated, because they are tissue constituents usually present at the neck of the bladder.

Only theoretic explanations have been offered for the origin of these mixed tumors. Heterologous mesoblastic tissues, often immature and occasionally with epithelial elements, suggest that embryonic tissues in some way have been stimulated to growth. According to Kistler,[12] many authors have expressed the belief that these tissues arise from fetal rests (Cohnheim's theory). Wilms explained their presence on the basis of sclerotome or myotome tissues displaced in the posterior regions of the body by the caudal growth of the wolffian duct. Gruber,[13] however, considered this unnecessary, the only requisite being mesenchymal tissues with growth potentials displaced from the renal blastema. The appearance of multicentric foci of cartilage in the stroma of these tumors favors this view. Kistler, in serial sections of a mixed tumor of the uterus, found that the cartilage originated in discrete foci in the stroma. The smallest islets and the periphery of others were fibrous tissues. Toward the center of the masses the cartilage tissues were more clearly differentiated. The many discrete masses of cartilage arising in widely separated places in the tumor, Kistler stated, suggested that either there are multiple foci of cartilage cells scattered in the stroma from which islets develop or growth factors acting focally stimulate the formation of cartilage tissues. The latter seemed plausible because the islets of cartilage were not uniform in size and differed in their degree of differentiation. Gruenwald[14] stated that two kinds of abnormal developmental processes should be considered in determining the origin of tumor tissues not corresponding with normal tissues in a respective site, as exemplified by these mixed tumors. These are the occurrence of aberrant germs and the abnormal differentiation of cells in loco. The aberrant germs were originally part of a primordium but lost their position and are found later separated and distant from the mother tissues. The abnormal differentiation in loco occurs if the cells have developmental potencies which normally remain dormant. Persistence of normally degenerating organs or tissues, according to Gruenwald, is another possibility: namely, a time rather than a spatial factor.

SUMMARY

A pedunculated growth attached to the left side of the trigone of the urinary bladder in a man aged 83 years contained islets of cartilage and had other mesoblastic tissues characteristic of mixed tumors of the genitourinary system. Only a few reports of such cancerous mixed tumors of the bladder have been published.

12. Kistler, G.: Am. J. Cancer **16**:399, 1932.
13. Gruber, cited by Hückel.[2]
14. Gruenwald, P.: Arch. Path. **36**:190, 1943.

DEVELOPMENT OF CARDIAC LESIONS IN THIAMINE-DEFICIENT RATS

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In a recent study [1] of the effect of ingestion of alcohol on the thiamine requirement of rats we observed that 4 of the animals had postmortem evidence of cardiac failure. The hearts were enlarged, and the thoracic and peritoneal cavities were filled with a clear colorless fluid. Histologic examination of 3 of these hearts revealed lesions of the auricular myocardium. The auricles of the fourth were not present in the sections examined. By manipulation of the experimental conditions, a simple method was found by which cardiac lesions could be produced in the majority of the thiamine-deficient rats.

EXPERIMENTAL PROCEDURE

Weanling albino rats of the NIH strain were used. They were housed individually in wide mesh, galvanized wire cages that were elevated to prevent coprophagy. All the rats drank water and ate diet 461, which had the following composition: leached and alcohol-extracted casein, 18; sucrose, 73; cottonseed (Wesson) oil, 3; cod liver oil, 2; Osborne and Mendel salt mixture, 4. The rats that served as controls received a daily supplement of 50 micrograms of riboflavin, 50 micrograms of calcium pantothenate, 20 micrograms of pyridoxine, 1 mg. of nicotinic acid, 20 mg. of choline chloride and 100 micrograms of thiamine hydrochloride in 1 cc. of distilled water. This was given to the rats in a separate supplemental cup. For six weeks the rats on the thiamine-deficient regimen were given a similar daily supplement that contained only 4 micrograms of thiamine hydrochloride. After this preliminary six week period, the thiamine compound was withdrawn completely. When signs of acute thiamine deficiency—spasticity, ataxia and convulsive seizures—developed, 50 micrograms of thiamine hydrochloride in 0.5 cc. of distilled water was injected subcutaneously. The thiamine-deficient dietary regimen was then continued until another episode of acute symptoms occurred. At this time 50 micrograms of thiamine hydrochloride was given as before. This procedure was repeated until the rats died or until they were killed. The control rat for each litter was killed when the last rat of that litter was dead.

In part I, 20 litters of 4 rats each were used. Litter mates were of the same sex. One rat from each litter served as the control, and the other 3 were kept on the thiamine-deficient regimen. The rats ate diet 461 ad libitum. In part II, 24 pairs of litter mates of the same sex were used. One rat of each pair was a control, and the other rat was on the thiamine-deficient regimen. The rat on the thiamine-deficient regimen ate the diet ad libitum. The paired control was given the same amount of food as the deficient rat.

Autopsies were done on all control and 82 of the 84 deficient rats. Tissue for histologic examination was fixed in 4 per cent solution of formaldehyde, embedded in paraffin and stained routinely by polychrome methylene blue eosinate and by the Van Gieson method for demonstrating collagen. Preparatory to embedding, the hearts were hemisectioned in a plane passing through all four chambers. In most cases both halves of the heart were embedded and sections made from each block. In certain cases, mentioned later, numerous sections of the heart were examined. In the early part of the study, histologic examination was done on most of the viscera. Later, only the heart, the lungs and the liver were examined since significant lesions were not found in the other organs. The brain, the spinal cord and a

From the divisions of pathology and chemotherapy of the National Institute of Health.

1. Lowry, J. V.; Sebrell, W. H.; Daft, F. S., and Ashburn, L. L.: J. Nutrition **24**:73, 1942.

sciatic nerve of each of 3 rats, kept on the deficient regimen for one hundred, one hundred and forty-five and one hundred and eighty-seven days, were studied histologically. Since pathologic alteration was not found, further reference to the nervous system will not be made.

RESULTS: PART I

All except 3 of the 60 rats fed the diet deficient in thiamine died after having been subject to experiment for thirty-three to one hundred and forty-five days—average, sixty-three days. Three were killed after one hundred, one hundred and forty-five and one hundred and eighty-seven experimental days. Eighteen rats died without having been observed in an episode of acute symptoms. The remainder, when death occurred, had passed through from one to six such episodes.

Gross Examination.—Nine rats had 1 to 2 cc. of fluid in the thoracic cavity. Two of these had fluid in the abdomen also. The fluid was clear except in 1 rat; in this the fluid was of milky appearance. Many hearts were enlarged, with moderate to marked dilatation of the right auricle. In some cases the left auricle was similarly dilated, but to a much less degree. An occasional heart also showed slight dilatation of the right ventricle. There was no alteration in the walls of the heart that could be recognized by gross examination.

Microscopic Examination.—(*a*) Heart: Histologic lesions were found in 46 of 58 hearts of the thiamine-deficient rats. (Two rats were markedly autolyzed and not examined.) The lesions occurred almost exclusively in one or both auricles, ventricular lesions being seen in only 7 hearts. The 12 hearts in which pathologic alteration was not found showed moderate autolysis. However, the autolysis was not of such grade as to prevent recognition of lesions other than of a very early stage.

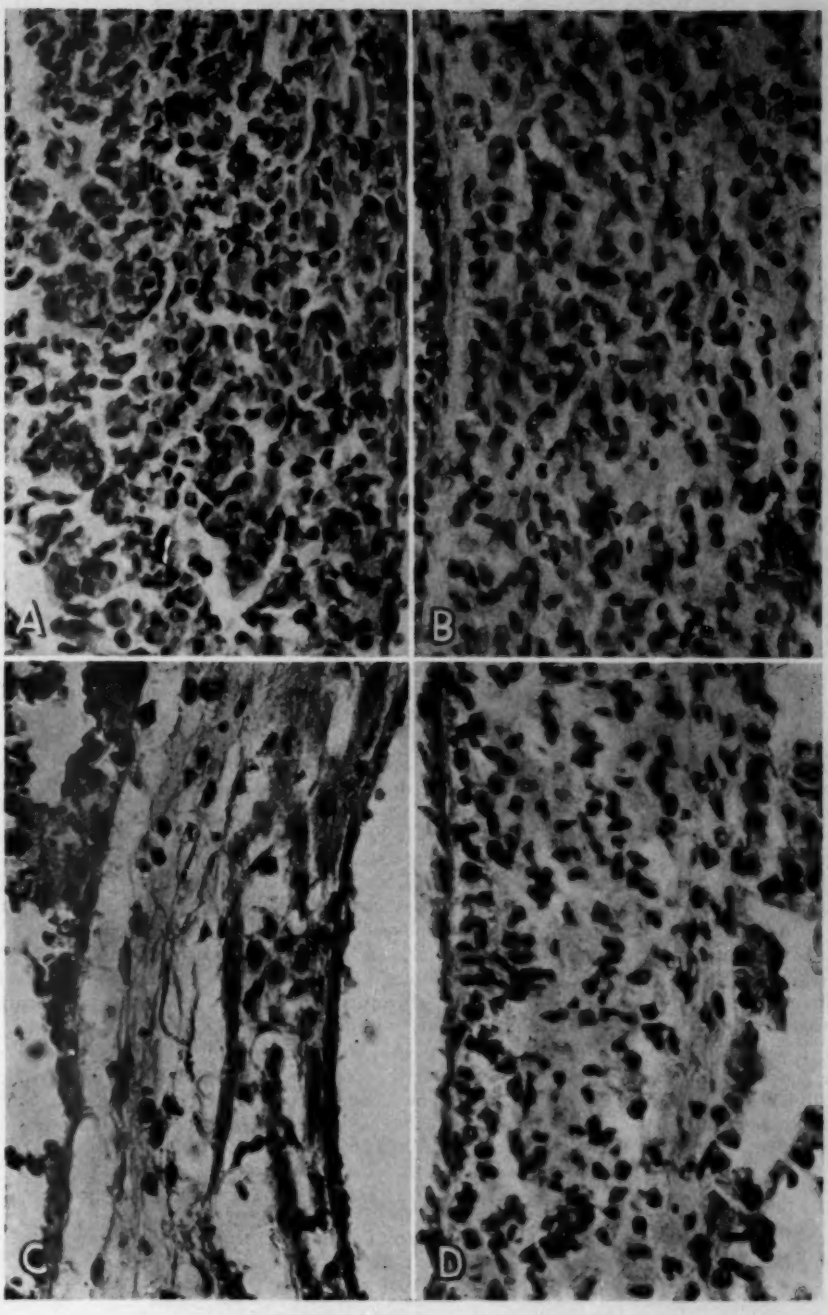
For purposes of description the cardiac lesions may be divided into three stages: (1) necrosis with cellular infiltration, (2) interstitial proliferation and (3) inactive stage. In the first stage the muscle fibers were hyalinized and showed increased oxyphilia of their cytoplasm and pyknosis, karyorrhexis or absence of their nuclei. Some necrotic fibers were seen in which the form was preserved; others were fragmented, and in some instances only oxyphilic debris and nuclear fragments remained. In an occasional area of involvement a few fibers appeared normal, but generally all fibers in such an area were in some stage of degeneration. Slight to moderate cellular infiltration was seen in most lesions. The cells comprised neutrophils, lymphocytes and large mononuclear cells in varying proportion. These infiltrating cells were generally less numerous in the early phases of the process; however, in a few hearts focal cellular infiltration was seen without recognizable alteration of muscle fibers. Sometimes muscle fibers were granular or exhibited vacuolation and swelling with dispersion of fibrillae.

In the proliferative stage, muscle fibers usually were absent. However, in a few cases a little oxyphilic debris or remnants of muscle fibers were recognized, most often at the peripheral margins of the lesions. The proliferation of fibroblasts was the most striking feature and varied in degree from slight to marked. In areas showing the most marked proliferation the entire thickness of the auricular wall was formed of compactly disposed plump fibroblasts. At the other extreme, foci were seen in which only a few fibroblasts were present, the auricular wall being formed of a loose connective tissue network. Rarely, increased numbers of fibroblasts were seen in small areas showing no loss of, or damage to, muscle fibers. In this stage large mononuclear cells were present, usually in small numbers; less frequently lymphocytes and neutrophils were seen. In some lesions seen in this stage a few intercellular collagen fibers were present.

In the inactive stage the auricular wall showed moderate to marked decrease in the number of muscle fibers. In many cases focal, often large areas showed absence of muscle fibers with marked thinning of the wall. Inactive lesions usually showed some fibrosis, but in most cases it was of slight degree. In some lesions the apparent fibrosis appeared to be a result of condensation of stroma following disappearance of muscle rather than an actual increase in collagen fibers. In a few instances the fibrous tissue was present in such amounts that the auricular wall was approximately normal in thickness although muscle fibers were absent. Generally this fibrous tissue was only sparsely cellular.

Mural thrombi were found in the auricles of 3 hearts; in 1 the involvement was bilateral, while in the other 2 the thrombi were in the left auricle only. One thrombus was adherent to, and caused fixation of, the lateral leaflet of the mitral valve. Two of these hearts also showed single small round thrombi in the pulmonary vein just distal to the auricle. One thrombus was partly organized and focally calcified.

The auricles of 6 hearts were sectioned serially and every twentieth section examined. In addition, one or both halves of 14 hearts were sectioned serially for a depth of 0.5 mm. and every tenth section examined. From this fairly extensive sampling it was evident that



A, interauricular septum showing almost complete replacement of muscle fibers by large mononuclear cells. A few non-nucleated fragments of hyalinized muscle fibers are seen along the upper right margin. Hematoxylin and eosin stain; × 385.

B, auricular wall formed of compactly disposed plump fibroblasts. Muscle fibers are absent. Azure eosinate stain; × 415.

C, segment of pulmonary vein within lung. Part of three alveoli are seen along the left margin. The wall of the vein shows necrosis of muscle fibers, with early cellular infiltration. Two surviving muscle fibers are seen in the upper right area. Hematoxylin and eosin stain; × 385.

D, segment of a pulmonary vein within a lung. The wall of the vein is formed of fibroblasts and shows slight cellular infiltration. Muscle fibers are absent. Hematoxylin and eosin stain; × 465.

all portions of the auricles were susceptible to injury in thiamine deficiency. Lesions were found in the interauricular septum, the main body of the auricle and in the appendages. Often the musculi pectinati of the appendages were severely damaged. Frequently they were present in their normal thickness but almost completely devoid of muscle fibers, a loose reticulum serving to maintain their form.

The extent of the pathologic alteration varied in different hearts and often in the two auricles of the same heart. In some a single small lesion was seen in only one auricle whereas in a few both auricles showed subtotal involvement. The auricular lesions were bilateral in 32 hearts. The extent of involvement of the two auricles was approximately the same in 21 hearts, greater in the right in 5 and in the left in 6. The extent of the injury was approximately the same in those cases in which there was fluid in the chest or the abdominal cavities (as a group) as in those in which no fluid was found at autopsy.

In 21 hearts in which the involvement was approximately of the same degree in both auricles, this involvement was slight in 3, moderate in 8 and marked in 10. In the cases in which only one auricle showed lesions, there was no significant difference in the incidence with which the right or the left auricles were affected. Of 44 altered hearts in which both auricles were examined, the right was involved in 5, the left in 7 and both in 32.

Often lesions of varying ages were observed in the same heart and occasionally in the same auricle. As would be expected, however, such combinations of lesions were less frequent in those animals which had been on experiment for the shortest periods. Of 22 animals which had been on experiment for fifty-two days or less, 18 showed only lesions in the stage of necrosis or proliferation, whereas of 24 animals on experiment for more than fifty-two days, lesions of the necrosing or the reparative stage existing alone occurred in only 12.

Lesions of the ventricular myocardium were seen in only 7 hearts. Three of these were found only after examination of multiple sections, as described in an earlier paragraph. In all the involvement was minimal, and in 5 only single minute lesions were observed. They showed either necrosis of muscle fibers or interstitial proliferation or both. Evidence of previous damage (scarring) was not found.

(*b*) Lung: The pulmonary veins of the rat are formed largely of striated muscle of the cardiac type. However, within the lung this muscle is seen only in the main branches or their larger radicles. In these pulmonary veins were seen lesions which were similar in all respects to those described in the auricles. The involvement was segmental whether the vessels were observed in cross or in longitudinal sections. Frequently, most muscle fibers had disappeared from the greater portion of the wall of the vein, and there was slight, rarely moderate associated fibrosis. In a number of veins the walls were very thin and appeared to be formed entirely by the condensed or collapsed stroma.

Of 55 rats whose lungs were examined, lesions of the pulmonary vein were seen in 21. In 19 of these rats the hearts also were involved. In some cases the heart sections included part of the pulmonary veins just distal to the auricles. These usually showed pathologic alteration similar to that present in the veins within the lung.

(*c*) Liver: The livers from 24 rats were examined. Three of these rats showed neither cardiac nor hepatic alteration. Of 21 with cardiac lesions, 9 showed slight, rarely moderate congestion of the liver; in most of these the congestion was centrolobular, but in only 1 was there slight atrophic narrowing of the hepatic cords in this area. In 2 of those showing congestion an occasional necrotic liver cell was seen.

CONTROLS

The 20 control rats which received adequate amounts of thiamine did not show lesions of the heart, the pulmonary vein or the liver such as those described.

RESULTS: PART II

Rats on a diet deficient in thiamine gain weight slowly and at death are usually emaciated. This is due largely to the fact that only a small amount of food is consumed. To determine the effect, if any, of inanition on the production of cardiac lesions a paired feeding experiment was set up as described in an earlier paragraph.

Inanition developed in the rats receiving adequate thiamine with a restricted intake of food, as well as in the rats on the thiamine-deficient regimen.

Ten of the thiamine-deficient and 4 of the inanition control rats died during the experiment. The remaining 14 thiamine-deficient and 20 control rats were killed after having been on experiment from forty-seven to one hundred and thirty days.

None of the 24 inanition control rats showed either gross or microscopic evidence of damage to the myocardium.

One of the thiamine-deficient rats had fluid in the pleural cavities, and 2 had mural thrombi in the heart. Microscopically, these rats showed pathologic changes similar in all respects to those described in detail in part I. The incidence of involvement, however, was lower in this smaller series. Lesions of the auricular myocardium were found in 11 of the 24 rats. Two of these showed slight focal ventricular involvement. Ten of the 11 rats also showed pathologic alteration of the pulmonary veins. In addition there were 4 rats with involvement of the pulmonary vein but without lesions of the heart.

COMMENT

Lesions of the myocardium have been described in comparatively few of the studies on vitamin B_1 deficiency. Porto and de Soldati [2] in studying the hearts of 4 dogs on a thiamine-deficient diet found focal hyalinization and necrosis in 2. One of these dogs had the lesion in the right auricle. These authors [3] also reported the occurrence of two areas of infarction of the left ventricle of another dog on a thiamine-deficient regimen. Swank [4] found myocardial lesions in a few pigeons fed a diet partially deficient in thiamine; Van Etten, Ellis and Madsen [5] described scattered areas of atrophy and necrosis in the hearts of 7 pigs fed a diet treated with sodium sulfite–sulfur dioxide to destroy thiamine, and Swank, Porter and Yeomans [6] reported the occurrence of focal necrosis of the myocardium in 3 of 14 thiamine-deficient dogs. In Chastek paralysis of foxes (due, at least in part, to thiamine deficiency) Evans, Carlson and Green [7] found myocardial degeneration in addition to other lesions. In a recent study on the effect of ingestion of alcohol on the thiamine requirement of rats, Lowry, Sebrell, Daft and Ashburn [1] described lesions in the auricular myocardium of 3 animals. In the areas of alteration, muscle fibers were decreased in number or were absent, and there were slight proliferation of fibroblasts, deposition of collagen and cellular infiltration; lesions were not found in the ventricles. Wintrobe and co-workers [8] in a study of thiamine deficiency in swine found extensive focal and diffuse myocardial necrosis in 6 of 9 pigs. The detailed report on the myocardial changes was made by Follis, Miller, Wintrobe and Stein.[9]

It appears that the lesions referred to in the report just cited are essentially similar; they differ only in extent, location and stage in which observed. Only in the reports of Porto and de Soldati,[2] Lowry and associates [1] and Follis and associates [9] were lesions of the auricular myocardium described. In the present observations the preponderant auricular involvement is a striking feature. Of 57 hearts showing pathologic alteration, all showed the auricles regularly involved while only 9 revealed ventricular lesions; the latter were usually single and small. From this it is evident that the auricles are more sensitive than the ventricles to a deficiency of thiamine. This conclusion is supported by the finding of lesions of the pulmonary veins, since the walls of these vessels in the rat are a continuation of the auricular myocardium. Follis and co-workers [9] also suggested that the auricles were particularly sensitive to this deficiency. Their statement was based on the fact that the auricular lesions in one pig were more severe than those of the ventricles and that in another pig the lesions were present only in the auricles.

2. Porto, J., and de Soldati, L.: Rev. Soc. argent. de biol. **15**:303, 1939.
3. Porto, J., and de Soldati, L.: Rev. Soc. argent. de biol. **15**:427, 1939.
4. Swank, R. L.: J. Exper. Med. **71**:683, 1940.
5. Van Etten, C.; Ellis, N. R., and Madsen, L. L.: J. Nutrition **20**:607, 1940.
6. Swank, R. L.; Porter, R. R., and Yeomans, A.: Am. Heart J. **22**:154, 1941.
7. Evans, C. A.; Carlson, W. E., and Green, R. G.: Am. J. Path. **18**:79, 1942.
8. Wintrobe, M. M.; Stein, H. J.; Miller, M. H.; Follis, R. H., Jr.; Najjar, V., and Humphreys, S.: Bull. Johns Hopkins Hosp. **71**:141, 1942.
9. Follis, R. H., Jr.; Miller, M. H.; Wintrobe, M. M., and Stein, H. J.: Am. J. Path. **19**:341, 1943.

In this connection it is interesting that Muus, Weiss and Hastings [10] in experiments on thiamine deficiency in rats found that there was a reduction from normal in the oxygen consumption of the auricular myocardium in vitro, while that of the ventricular muscle was normal.

It is certain that the cause of death of many rats was the occurrence of acute thiamine deficiency. A number of animals were observed to die with symptoms of an acute episode (spasticity, ataxia and convulsive seizures). Although we are unable to state definitely that any of the rats died of cardiac failure, the gross and microscopic changes in the heart together with the fact that 10 rats had pleural or abdominal effusion makes this a strong probability.

Since signs of acute thiamine deficiency can often be dramatically relieved by the administration of this vitamin, it is likely that a disturbance of cardiac function can occur before the development of histologically demonstrable alteration of muscle fibers. However, it would appear that the occurrence of extensive necrosis of the auricular myocardium would render irreversible any existing alteration of impulse initiation or conduction. Electrocadiographic studies by King [11] lend support to this belief.

Although a number of hearts were much enlarged, dilatation of one or more chambers, particularly of the right auricle, appeared to be an adequate explanation of the enlargement. Histologically there was no evidence of muscle hypertrophy. Cardiac lesions are not necessarily a late manifestation of thiamine deficiency, for of 11 rats which died during the six weeks of low intake of thiamine (previous to complete withdrawal) 7 had auricular lesions. Most of these were of moderate severity. In some rats the myocardial involvement had its inception at a much later period, for of 24 rats which had been subject to the experiment for fifty-three to one hundred and ten days 12 showed only recent lesions.

Schrader, Prickett and Salmon [12] and Follis, Orent-Keiles and McCollum [13] have reported the occurrence of myocardial lesions in rats fed potassium-deficient diets. Thomas, Mylon and Winternitz [14] found similar lesions in rats and hogs whose diets were deficient in both potassium and vitamin B_6. In the study presented here the rats received adequate amounts of both potassium and B_6. Also, the control rats receiving the same amounts of these substances but given 50 micrograms of thiamine hydrochloride daily did not show cardiac injury.

In the histologic study of the human heart in beriberi, fatty degeneration and vacuolar or hydropic degeneration of muscle fibers have been noted. It appears that these observations are not constant, and the significance given them varies. Wenckebach [15] said that the fatty degeneration is generally considered to be without meaning, and Weiss and Wilkins [16] found hydropic degeneration in other than beriberi hearts. Focal lesions of the myocardium in beriberi have been rarely reported. Dürck [17] found such lesions in 2 of 7 hearts examined histologically.

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10. Muus, J.; Weiss, S., and Hastings, A. B.: J. Biol. Chem. **129**:303, 1939.
11. King, W. D.: Alterations in the Cardiac Conduction Mechanism in Experimental Thiamine Deficiency, to be published.
12. Schrader, G. A.; Prickett, C. O., and Salmon, W. D.: J. Nutrition **14**:85, 1937.
13. Follis, R. H., Jr.; Orent-Keiles, E., and McCollum, E. V.: Am. J. Path. **18**:29, 1942.
14. Thomas, R. M.; Mylon, E., and Winternitz, M. C.: Yale J. Biol. & Med. **12**:345, 1940.
15. Wenckebach, K. F.: Das Beriberi-Herz; Morphologie, Klinik, Pathogenese, Berlin, Julius Springer, 1934.
16. Weiss, S., and Wilkins, R. W.: Ann. Int. Med. **11**:104, 1937.
17. Dürck, H.: Untersuchungen über die pathologische Anatomie der Beriberi, Berlin, Gustav Fischer, 1908.

Dock [18] in reporting 5 cases of beriberi stated that there were small patches of scarring in the left ventricle in 2 of these cases and that in 1 of these minute infarcts were also present. A few small scars were found in 1 of 2 beriberi hearts studied by Hussey and Katz.[19] Mural thrombi were present in the heart in all 5 cases reported by Dock and in both of the hearts studied by Hussey and Katz. It is difficult to determine from the literature how frequently the auricles have been examined histologically. However, in view of the fact that cardiac lesions in thiamine-deficient rats and swine are most frequent and extensive in the auricular myocardium, this part of the human heart should be subjected to more critical study in cases of beriberi.

SUMMARY

Sixty weanling rats were fed a purified diet containing or supplemented with adequate amounts of all the known required vitamins except thiamine. Thiamine hydrochloride was given in daily doses of 4 micrograms for a period of six weeks. The thiamine compound was then completely withdrawn until signs of acute deficiency appeared. At this time 50 micrograms of thiamine hydrochloride was given subcutaneously. The rats received no further thiamine until acute symptoms again appeared. This procedure was repeated until the animals died or were killed. Fifty-seven rats died after thirty-three to one hundred and forty-five (average, sixty-three) experimental days; 3 were killed after one hundred, one hundred and forty-five and one hundred and eighty-seven days. At autopsy most of these rats had enlarged hearts mainly because of dilatation of the right auricle. Fluid was present in the pleural or the abdominal cavities of 9 rats. Microscopically, the auricles of 47 hearts showed necrosis of muscle fibers, cellular infiltration and proliferation or evidence of previous damage, such as a decreased number or absence of muscle fibers and slight to moderate fibrosis. Similar lesions were seen in the ventricular myocardium in only 7 rats, and in these the involvement was minimal. Similar pathologic changes also occurred in the pulmonary veins of 21 rats.

Both auricles were involved in the majority of animals, and in those in which only one auricle showed lesions there was no significant difference in the frequency with which the right or the left auricle was affected. Mural thrombi were present in the left auricles of 2 hearts and in both auricles of a third. Two of these also showed single small thrombi in the pulmonary vein just distal to the auricle.

Cardiac lesions were not observed in control rats receiving 100 micrograms of thiamine hydrochloride daily and allowed to eat ad libitum. The results of a paired feeding experiment showed that inanition played no part in the development of cardiac lesions in the thiamine-deficient rats.

18. Dock, W.: Tr. A. Am. Physicians **55**:61, 1940.
19. Hussey, H. H., and Katz, S.: M. Ann. District of Columbia **11**:247, 1942.

RELATION OF THE POSTMORTEM INTERVAL TO THE SYNTHESIS OF GLYCOGEN FROM DEXTROSE BY SURVIVING LIVER

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Experiments by Cross and Holmes [1] and by Ostern, Herbert and Holmes [2] have shown that slices of rabbit liver will synthesize glycogen from dextrose when incubated in isotonic solution of three chlorides U. S. P. (Ringer's solution) enriched by calcium. Cross and Holmes [1] have found that the in vitro formation of carbohydrate from various substrates is reduced if the liver is poisoned by diphtheria toxin and that the in vitro synthesis of glycogen is abolished by diphtheritic toxemia and by the addition of epinephrine. In any case formation of carbohydrate could be demonstrated only when the initial carbohydrate content of the liver slices was low. In an investigation of conditions necessary for the synthesis of glycogen from dextrose by rat liver slices, Hastings and Buchanan [3] have found that glycogen is rapidly formed when the slices are incubated in a solution with cation concentrations comparable to those of intracellular fluid. Moreover, these authors have observed that if the initial level of glycogen in the liver is above 3 mg. per gram of tissue, there is no synthesis of glycogen even under conditions of optimal cation concentrations.

These experiments suggest an approach to the study of diseases as they occur naturally in man. It should be possible, by applying the same methods to surviving human liver, to detect changes in carbohydrate metabolism associated with disease. However, it is first necessary to determine whether or not such a study would be practicable. In all experiments cited, efforts had been made to provide optimal conditions for the synthesis; and the interval between the death of the experimental animal and the placing of tissue into suitable mediums had been kept at a minimum. It is well known that many enzyme systems, depending on their complexity, break down more or less rapidly after the death of the individual. Since it is usually impossible to obtain tissues from the human cadaver within the first few minutes after death, it was necessary to determine how long after death the ability of liver to synthesize carbohydrate is retained. The reaction studied was the formation of glycogen from dextrose. To provide conditions optimal in other respects, the rabbit was used as the experimental animal, and a medium comparable to intracellular fluid was selected. The rabbits were subjected to a preliminary period of starvation in order to reduce the initial level of glycogen in the liver. Our experiments have shown that the ability of rabbit liver slices to form glycogen from dextrose is rapidly lost after the death of the animal. The number of experiments is small, but the results were consistent and indicate that this method of study cannot be

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From the Department of Pathology, Washington University School of Medicine.

This investigation was carried out with the aid of a grant from the Commonwealth Foundation.

1. Cross, M. C. A., and Holmes, E.: Brit. J. Exper. Path. **18**:370, 1937.
2. Ostern, P.; Herbert, D., and Holmes, E.: Biochem. J. **33**:1858, 1939.
3. Hastings, A. B., and Buchanan, J. M.: Proc. Nat. Acad. Sc. **28**:478, 1942.

applied directly to human liver tissue obtained at the routine autopsy. For this reason, and because these experiments confirm those of Hastings and Buchanan [3] in that synthesis of dextrose is greatest in a medium comparable to intracellular fluid, we are reporting them.

MATERIALS AND METHODS

Thirteen Havana rabbits of an inbred line and ranging from 1 to 3 months of age were used. The animals were starved for twenty-four hours before use and were killed by intravenous injection of air. Samples of liver were removed either immediately or after periods of fifteen, thirty and sixty minutes. In order to simulate conditions of the human autopsy, the body was left intact until the time chosen for the removal of liver. In a few instances, liver from the same animal was used for study immediately and after periods up to thirty minutes post mortem. In these cases, the incision was closed to prevent drying of the liver, and the subsequent samples were taken from lobes which had not been disturbed. Twenty experiments were performed, as shown in table 1. In nine experiments the tissue was prepared immediately after death; in five a period of fifteen minutes was allowed to elapse, and there were three experiments each in which tissues were removed thirty and sixty minutes after death.

The determinations were carried out in much the same manner as in the experiments cited.[4] The excised liver tissue was kept moist in Ringer-Warburg solution [4a] while being sliced, and portions of sliced liver, weighed rapidly to within 5 mg. of 500 mg., were placed in Warburg flasks containing 4.0 cc. of medium. In two early experiments shown in table 1 the medium was isotonic solution of three chlorides U. S. P. with added sodium bicarbonate containing 1 per cent of dextrose. However, a solution comparable in cation concentrations to intracellular fluid [5] was found to be more suitable for the demonstration of glycogenesis and was used in all subsequent experiments. To 3.8 cc. of this solution 0.2 cc. of Ringer-Warburg solution containing dextrose in 20 per cent concentration was added in order to bring the concentration of dextrose to 1 per cent in a volume of 4.0 cc. The flasks containing mediums and tissue were equilibrated in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide for five minutes. After this they were incubated at 38 C. with shaking for two hours. In a single experiment the flasks were incubated for one hour. Control tubes were set up in the same way but were quenched with 2.0 cc. of 80 per cent potassium hydroxide at the time the others were placed in the Warburg apparatus. On the average, twenty minutes was required to prepare the tissues. Preliminary oxygenation in Ringer-Warburg solution was carried out in 1 case, designated in table 1. This procedure added about ten minutes to the time of preparation and did not increase the rate of glycogenesis. At the end of the incubation, 2 cc. of 80 per cent potassium hydroxide was added to each flask, and the flasks were placed in boiling water for fifteen minutes. Glycogen was determined by the method of Good, Kramer and Somogyi,[6] 5 cc. aliquots of the alkaline hydrolysate being used. Reducing sugars after acid hydrolysis were measured by the Shaffer-Hartmann-Somogyi method.[7] The experiments were carried out in duplicate. In the cases in which the same liver served for more than a single experiment, separate controls were necessary because of the rapid breakdown of hepatic glycogen post mortem.

RESULTS

The results of the twenty individual experiments are shown in table 1. Glycogen is expressed as milligrams per gram of wet tissue. Included is the total time which elapsed from the death of the animal to the start of incubation. Averages of the initial and final glycogen values in comparable experiments of

4. See footnotes 1, 2 and 3.

4a. The formula for the Ringer-Warburg solution is: sodium chloride, 6.882 Gm. per liter; potassium chloride, 0.183 Gm. per liter; calcium chloride, 0.272 Gm. per liter.

5. The formula was furnished by Dr. John M. Buchanan. It differs from that reported by Hastings and Buchanan [3] in that calcium is added and magnesium is omitted. The solution was of the following composition: calcium 10 millimols per liter, potassium 145 millimols, chloride 135 millimols, bicarbonate 40 millimols. The p_H after equilibration with 5 per cent carbon dioxide–95 per cent oxygen was 7.5.

6. Good, C. A.; Kramer, H., and Somogyi, M.: J. Biol. Chem. **100**:485, 1933.

7. Koch, F. C.: Practical Methods in Biochemistry, ed. 3, Baltimore, Williams & Wilkins Company, 1941, p. 151.

each group are shown in table 2. It will be seen that in a medium similar to intracellular fluid there was abundant synthesis of glycogen by rabbit liver when the liver was removed immediately after death. However, the ability of the liver slices to synthesize glycogen decreased so rapidly after death that it could not be demonstrated consistently in tissues removed fifteen or more minutes post mortem.

TABLE 1.—*Relation of the Postmortem Interval to Glycogenesis by Surviving Rabbit Liver Slices (Except where otherwise indicated, the tissues were incubated for two hours at 38 C. with shaking in a medium containing 1 per cent dextrose and with cation concentration similar to those of intracellular fluid)*

	Experiment	Age of Rabbit, Mo.	Time Between Death of Rabbit and Start of Incubation, Minutes	Initial Glycogen Content, Mg. per Gm.	Final Glycogen Content, Mg. per Gm.
A. Tissue removed immediately after death					
	1	2	15	0.12	0.47*
	2A	2	18	0.96	0.47*
	2B	2	13	0.96	2.65
	3A	2½	17	0.32	3.86
	3B	2½	17	0.32	1.45†
	7	2	15	0.38	1.38
	7A	2	25	0.21	0.93§
	9	1½	18	0.40	2.50
	13A	1½	17	0.27	2.12
B. Tissue removed fifteen minutes after death					
	7B	2	30	0.39	0.95
	10	1	34	0.78	0.75
	11	1	33	0.12	0.71
	12A	1	32	0.19	0.08
	13B	1½	28	0.15	0.27
C. Tissue removed thirty minutes after death					
	8	2	52	0.08	0.17
	12B	1	64	0.14	0.17
	13C	1½	58	0.18	0.18
D. Tissue removed sixty minutes after death					
	4	2½	77	1.00	0.18
	5	3	76	0.17	0.17
	6	1	72	0.07	0.11

* The medium was a solution of three chlorides U. S. P. with added sodium bicarbonate containing dextrose.
† The preparation was incubated one hour.
§ The tissue was removed immediately after death but was oxygenated in Ringer-Warburg solution.

TABLE 2.—*Averages for Experimental Groups of Table 1 (the table includes only the results of the incubation of rabbit tissue slices for two hours in intracellular medium containing 1 per cent dextrose)*

Time After Death, Minutes	Experiments	Initial Glycogen Content, Mg. per Gm.	Final Glycogen Content, Mg. per Gm.	Average Change, Mg. per Gm.
0	5	0.47	2.50	+2.03
15	5	0.33	0.75	+0.42
30	3	0.13	0.17	+0.04
60	3	0.41	0.15	−0.26

There was an inverse relation between the amount of glycogen synthesized in two hours and the length of the period after death during which the tissue was left in the body. Of the two experiments shown in table 1 in which the medium was isotonic solution of three chlorides U. S. P. with added sodium bicarbonate containing dextrose, there was relatively little synthesis of glycogen in one and loss of glycogen in the other.

COMMENT

Our results agree with those of Cross and Holmes [1] in that rabbit liver slices will synthesize glycogen from dextrose in isotonic solution of the three chlorides U. S. P. with added sodium bicarbonate. However, synthesis was observed to take place to a greater extent in a medium comparable in cation concentrations to intracellular fluid. In this respect our results confirm those of Hastings and Buchanan,[3] although in their studies rat liver was used. Since rat liver, according to Lowry and Hastings,[8] will not synthesize significant amounts of glycogen from dextrose in isotonic solution of three chlorides with added sodium bicarbonate, the optimal conditions for our purposes seemed to be the use of rabbit liver and of a medium comparable to intracellular fluid. Even so, the synthesis of glycogen could not be demonstrated consistently if fifteen or more minutes had elapsed between the death of the animal and the removal of tissue for study. This method would therefore be applicable to the study of human liver tissue obtained at autopsy only if the cadaver could be examined within the first few minutes after death.

Of practical consideration is the initial level of glycogen in the liver. Cross and Holmes [1] stated that if the initial carbohydrate content of the liver slices is high, little or no additional carbohydate is formed during incubation. Hastings and Buchanan [3] found that glycogenesis by slices of rat liver will not take place if the liver contains more than 0.3 per cent of glycogen. In several experiments shown in table 1 a relatively high initial level of glycogen seemed to inhibit synthesis of glycogen by slices of rabbit liver. Since this relation may be assumed to hold for the livers of other species, it is important to know what levels of glycogen are to be expected in human livers obtained at routine autopsies. In a series of 55 human livers removed between forty minutes and two hours after death we have found that the glycogen content ranged from 0.005 to 5.0 per cent. The livers in 27 cases, or 49 per cent of the series, contained less than 0.3 per cent of glycogen. Presumably one half of the livers examined at routine autopsies would be suitable for this type of study if the tissue could be removed within a few moments after death.

Although a high glycogen content would preclude in vitro studies of glycogenesis in many human livers obtainable at autopsy, yet under certain conditions the glycogen content may be taken as an index of the ability of the liver to form glycogen. Two cases from the series of human autopsies described in the foregoing paragraph illustrate the possibilities of this approach. The patients, women aged 49 and and 46 years, respectively, died twenty-four and eighteen hours after major neurosurgical operations. Each had received large amounts of dextrose intravenously during the postoperative period. The first patient's liver appeared normal and contained 4.0 per cent of glycogen. The other patient exhibited the hepatorenal syndrome, and large areas of fatty metamorphosis, necrosis and hemorrhage were found in the liver. A trace only of glycogen, 0.005 per cent, was present. This liver was evidently unable to synthesize glycogen at a normal rate. Inasmuch as the conditions under which glycogenesis can be demonstrated in vitro are narrowly restricted, this phase of carbohydrate metabolism may be more profitably studied in relation to human disease by comparing the glycogen content of the liver post mortem with the amount of carbohydrate available to the liver during the period before death.

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8. Lowry, O. H., and Hastings, A. B.: Histochemical Changes in Ageing, in Cowdry, E. V.: Problems of Ageing, ed. 2, Baltimore, Williams & Wilkins Company, 1942, p. 728.

SUMMARY

The ability of surviving slices of rabbit liver to synthesize glycogen from dextrose when incubated in a medium comparable to intracellular fluid was found to decrease rapidly after death. Glycogenesis was not consistently demonstrated if fifteen or more minutes had elapsed post mortem before the tissues were removed from the animal. A relatively high initial level of glycogen in the liver seemed to inhibit glycogenesis in vitro.

It is concluded that this method of study is not generally applicable to human liver tissue in cases of disease, because tissue can rarely be obtained from the cadaver during the first few minutes after death and because a high initial glycogen content of the liver may be expected to inhibit further glycogenesis in vitro. A correlation of the glycogen content of the human liver post mortem with the amount of carbohydrate available to the liver during life is suggested as a more suitable approach to the study of this phase of hepatic function in human disease.

EFFECT OF ESTROGENS ON THE TESTIS IN HEPATIC INSUFFICIENCY

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Kyrle,[1] in 1909, and Weichselbaum,[2] in 1910, observed varying degrees of testicular atrophy in 35 cases of Laennec's cirrhosis and stressed the important role of chronic alcoholism and not cirrhosis in the genesis of the testicular changes. Corda[3] also observed testicular atrophy and gynecomastia in patients with cirrhosis of the liver. Silvestrini[4] noted fibrosis and "an inflammatory process" in the testes from 3 patients with cirrhotic livers and described associated hyperplasia of the mammary acinar epithelium. He ascribed these changes to an endocrine imbalance.

Evidence that an excess of estrogens is a most likely causative factor in the production of testicular atrophy and gynecomastia in persons with cirrhosis of the liver has recently been forthcoming from several sources. Many observers[5] have recognized that the administration of estrogens results in testicular atrophy. Zondek,[6] however, in 1934 was the first to demonstrate that the liver is capable of inactivating estrogens. The next important advance came in 1939 with the discovery by Glass, Edmonson and Soll[7] of increased amounts of free estrogens in the urine of males with cirrhotic livers. One is therefore led to the hypothesis that in males with hepatic insufficiency estrogens normally present in the blood stream are not inactivated and so accumulate in amounts sufficient to produce an estrogenic effect, namely, gynecomastia and testicular atrophy.

The present work was undertaken with a twofold purpose, first, to investigate the state of human testes in various conditions of hepatic insufficiency and, second, to test by experimental means the validity of the hypothesis that an excess of estrogens is active in the production of the testicular change. The first phase of the work is a study of the testis in Laennec's cirrhosis and in other states of hepatic failure. In addition to determining the incidence and the extent of testicular degeneration, it was hoped that one might in a detailed study of the pathologic changes evaluate the possible factors operating to produce these changes. In this connection, the questions of excess estrogens, hepatic failure and alcoholism were considered.

METHODS

Seventy-eight cases of Laennec's cirrhosis were available for study from the autopsy files of the Long Island College of Medicine and the Long Island Division of Kings County Hospital. Of these, 50 were excluded because of sex or diseases such as syphilis, tuberculosis, hydrocele, varicocele or mumps. Elimination of these cases left a selected group of 28 cases of cirrhosis in which no known factor other than senility was operating to produce testicular degeneration. To eliminate the age factor, three control testes were selected from persons of a similar age group, and the groups were studied and compared. The only criteria for the selection of the control cases was the absence of the diseases just listed, known to predispose to testicular atrophy.

Thirty-four cases comprised the "hepatic insufficiency" group. These included cases of primary and secondary carcinoma of the liver, hemochromatosis, "cardiac" cirrhosis, biliary obstructive cirrhosis, hepatic amyloidosis, and carcinoma of the head of the pancreas with obstructive jaundice. The latter group was included in view of the recent evidence of Cantarow and his co-workers[8] that estrogens are excreted in the bile.

The decision as to whether significant hepatic failure was present was frequently difficult to make. It was based on a consideration of the extent and the nature of the morphologic changes and as much clinical information as was available. Hyperchromic macrocytic anemia, changes in the albumin-globulin ratio and the icterus index were utilized as indexes of hepatic function. In this group, as in the cases of Laennec's cirrhosis, hepatic failure was the only factor other than age which might produce testicular atrophy and fibrosis.

TESTICULAR ATROPHY AND LAENNEC'S CIRRHOSIS

The most constant pathologic condition in the testes in cases of Laennec's cirrhosis was atrophy of the germinal epithelium. This was accompanied by a notable decrease in the size of the seminiferous tubules themselves (fig. 1 *B*). In 3 cases (3, 6 and 9) the diameters of seminif-

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1. Kyrle, W.: Verhandl. d. deutsch. path. Gesellsch. **13**:391, 1909.
2. Weichselbaum, A.: Verhandl. d. deutsch. path. Gesellsch. **14**:234, 1910.
3. Corda, L.: Minerva med. **5**:1067, 1925.
4. Silvestrini, R.: Riforma med. **142**:701, 1926.
5. Herrmann, E., and Stein, M.: Wien. klin. Wchnschr. **29**:177, 1916. Steinach, E., and Kun, H.: Biol. generalis **2**:815, 1926. Moore, C. R., and Price, D. R.: Am. J. Anat. **50**:13, 1932.
6. Zondek, B.: Skandinav. Arch. f. physiol. Chem. **70**:133, 1934.
7. Glass, S.; Edmonson, H., and Soll, S.: Endocrinology **27**:749, 1940.
8. Cantarow, A.; Rakoff, A.; Paschkis, K., and Hansen, L.: Proc. Soc. Exper. Biol. & Med. **49**:707, 1942.

erous tubules were measured, and the mean diameter of 100 tubules in each case was found to be 129 microns in case 3, 118 microns in case 6, and 150 microns in case 9, compared with the normal diameter of 150 to 300 microns.

TABLE 1.—*Age Grouping of Cases of Laennec's Cirrhosis*

Patient's Age	Total Number of Cases	Number with More Atrophy of Testis Than Controls	Percentage Showing More Atrophy of Testis Than Controls
30-40	3	3	100.0
40-50	7	6	85.7
50-60	10	3	30.0
60-70	4	1	25.0
Over 70	4	3	75.0
Total	28	16	57.1
Below 50	10	9	90.0
Over 50	18	7	38.9

definite hyperplasia was evident. The demonstration by Warren and Olshausen [9] that this change is present in a great variety of conditions makes its significance difficult to evaluate. It is interesting, however, to note that Gardner [10] reported hyperplasia of Leydig cells in mice following administration of an estrogen.

The results obtained by comparing the testes in cases of Laennec's cirrhosis individually with the three age controls are given in table 1. In order for testicular atrophy in the cases of cirrhosis to be considered as significant, the changes had to be appreciably more pronounced than in all three age controls. The recorded percentages of cases with significant atrophy of the testes are therefore considerably lower than those which would have resulted had just atrophy of the testes been noted in the cases of cirrhosis. As the cases were studied, it became evident that

TABLE 2.—*Cases of Laennec's Cirrhosis*

Autopsy	Patient's Age	Color Index	Albumin-Globulin Ratio	Icterus Index	History of Alcoholism	Atrophy of Testis
1	34	1.19	2.4:3.7	16.5	Heavy drinker past 17 years	++
2	35	0.96	.....	..	None	++
3	37	1.12	.....	7	Heavy drinker	+++
4	42	....	1.6:4.7	30	One quart sherry a day	+++
5	43	0.98	2.3:4.9	12	None	++
6	44	0.88	1.6:4.6	20	Definitely no alcohol	+++
7	44	....	.....	..	Chronic alcoholism	+
8	46	1.19	.....	71	One pint whisky a day for 20 years	+
9	46	0.94	2.2:4.5	32	"Hard liquor" for 20 years	+++
10	49	0.64	.....	..	No alcoholism	+++
11	51	0.86	.....	..	"Moderate"	None
12	52	....	.....	..	None	+
13	55	....	.....	..	Heavy consumer of beer and wine	+
14	56	1.24	2.2:3.4	80	Heavy drinker for 30 years	+++
15	57	0.99	.....	..	Heavy drinker until 2 years ago	+
16	59	0.93	.....	34	Heavy drinker for years	+
17	59	0.76	.....	..	None	+
18	59	1.08	.....	..	Heavy drinker	++
19	59	1.07	3.5:2.8	..	One quart whisky a day for 20 years	+
20	59	....	3.2:2.6	66.8	Wine in youth, none lately	+
21	60	0.85	3.1:2.6	..	None	+
22	63	1.08	.....	..	None	+
23	63	0.63	.....	..	None	+
24	65	0.96	3.2:3.0	10	One quart a day for years	+
25	70	1.31	.....	..	Heavy drinker (bad quality)	+++
26	70	....	.....	..	None	++
27	80	0.91	.....	..	None	+++
28	83	0.95	.....	..	None	+++

The atrophy appears even more pronounced if one considers that approximately one third of the measured diameters consisted of thickened basement membrane.

Interstitial fibrosis to a variable degree accompanied the tubular atrophy but could nowhere be described as severe. Throughout the germinal line of spermatogenesis, pyknosis and karyolysis were frequently observed, as well as marked cytoplasmic vacuolation. The Sertoli cells were at times the only cellular elements visible in the tubules. These changes were in no way peculiar to the atrophy associated with cirrhosis of the liver and could not be differentiated from similar changes in atrophy associated with other diseases.

The interstitial cells of Leydig showed no definite changes except in 2 cases in which the incidence of testicular atrophy among patients with hepatic cirrhosis was higher in the younger age groups. The cases were therefore tabulated according to the various decades of life (table 1).

The contrast in the effect of hepatic cirrhosis on the testis in those below as opposed to those above the age of 50 years is evident. Not only was the incidence of testicular atrophy greater in the younger age groups but the lesions themselves were as a rule more severe than those in the older age group. The significance of this difference in the behavior of the different age groups is difficult to interpret. The data appear to suggest that some changes occur in the endocrine balance in men at about the age of 50 years.

9. Warren, S., and Olshausen, K.: Am. J. Path. **19**:307, 1943.

10. Gardner, W. U.: Anat. Rec. **68**:339, 1937.

TESTICULAR ATROPHY AND HEPATIC INSUFFICIENCY ASSOCIATED WITH CIRRHOSIS OF THE LIVER AND CHRONIC ALCOHOLISM

The detailed data regarding the state of hepatic function and the history of alcoholism are presented in table 2. The history of alcoholism is noted in view of the long-standing impression that alcohol per se is capable of producing testicular degeneration and that it is related to hepatic damage. To facilitate comparison, the degree of testicular atrophy is expressed as none, 1 plus, two plus or three plus. The latter designations connote correspondingly slight, moderate and severe degrees of tubular atrophy and fibrosis (table 2).

Several facts become apparent on surveying the data in table 2. The first is that the incidence of alcoholism is high. A certain proportion of this high incidence may be accounted for by the methods of history taking, more attention probably being devoted to the alcoholic history in cases of suspected Laennec's cirrhosis than was usual in the routine procedure. This is suggested by the greater detail available concerning the alcoholism among the patients with cirrhotic livers.

From the data it is apparent that there is no direct causal relation between alcoholism and atrophy of the testis. Thus, among the cases of cirrhosis 7 instances are found in which there was no significant testicular atrophy in the presence of a definite history of alcoholism. Conversely, there are 6 cases in which there was "moderate" or "severe" testicular atrophy in the absence of alcoholism. In the latter cases the presence of hepatic failure as determined either by laboratory data or by morphologic changes is the only feature accounting for the testicular changes. Further, the only instance among the 10 patients whose age was below 50 years in whom significant testicular atrophy was absent is that of a 46 year old patient with cirrhosis who had a long-standing history of severe alcoholism.

There are numerous instances among the cases presented which illustrate the coexistence of hepatic failure and atrophy of the testes. The albumin-globulin ratio was available in only 10 cases, but the severest degrees of testicular atrophy, i. e., 2 or 3 plus, were seen only in those 6 cases showing a reversal of the albumin-globulin ratio. The remaining 4 cases of cirrhosis presented no reversal of ratio, and each showed only slight testicular change.

TESTICULAR ATROPHY AND HEPATIC INSUFFICIENCY ASSOCIATED WITH HEPATIC DISEASE OTHER THAN LAENNEC'S CIRRHOSIS

A total of 34 cases were included in this group, among which were 9 cases of carcinoma of the head of the pancreas with obstructive jaundice, 2 cases of primary carcinoma of the liver, 15 cases of secondary carcinoma of the liver, 2 cases of hemochromatosis, 3 of "cardiac" cirrhosis, 2 of biliary obstructive cirrhosis and 1 case of hepatic amyloidosis. No effort was made to compare cases with age controls, but an attempt was made to correlate the degree of testicular atrophy with that of hepatic damage.

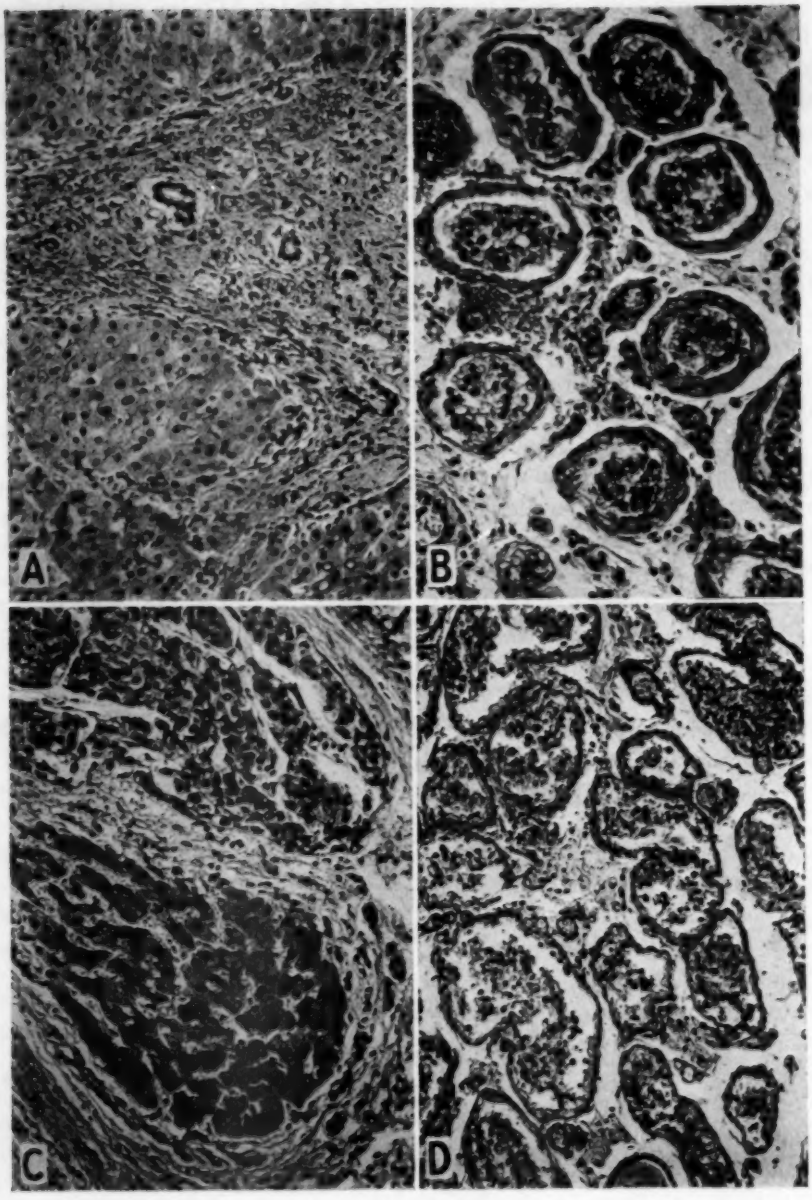
The results obtained were inconstant and therefore are not given in tabular form, for consideration was made difficult by the fact that laboratory data regarding the state of hepatic function were frequently meager. The only correlation between damage of the liver and testicular atrophy was seen in 2 cases of primary carinoma of the liver, in both of which this disease was associated with extensive cirrhosis, and in the 1 case of amyloidosis (figs. 1 *C* and *D* and 2 *A* and *B*). In the 3 patients, aged 51, 58 and 46 years, respectively, the liver was almost completely replaced by tumor tissue, connective tissue or amyloid and the testes showed definite tubular atrophy and slight interstitial and tubular fibrosis; there was also laboratory evidence of hepatic failure.

The testes in cases of metastatic carcinoma of the liver frequently showed only slight atrophy in spite of senility, extensive replacement of liver tissue by tumor, chronic illness and chronic inanition associated with death from carcinoma. The cases of biliary and "cardiac" cirrhosis showed no constant tendency toward the production of testicular degeneration. Of the 9 cases of carcinoma of the head of the pancreas with obstructive jaundice, only 2 showed moderate or severe testicular atrophy, in spite of the fact that all the 9 patients were over the age of 50 and had carcinoma. The conclusion reached from surveying this group was that severe, extensive and long-standing hepatic damage must be present before any correlation can be established between it and atrophic changes in the testis.

TESTICULAR ATROPHY AND HEMOCHROMATOSIS

There is indirect evidence that a deranged endocrine balance exists in males with hemochromatosis. Sheldon [11] in his review of 311

11. Sheldon, J.: Lancet **2**:1031, 1934.



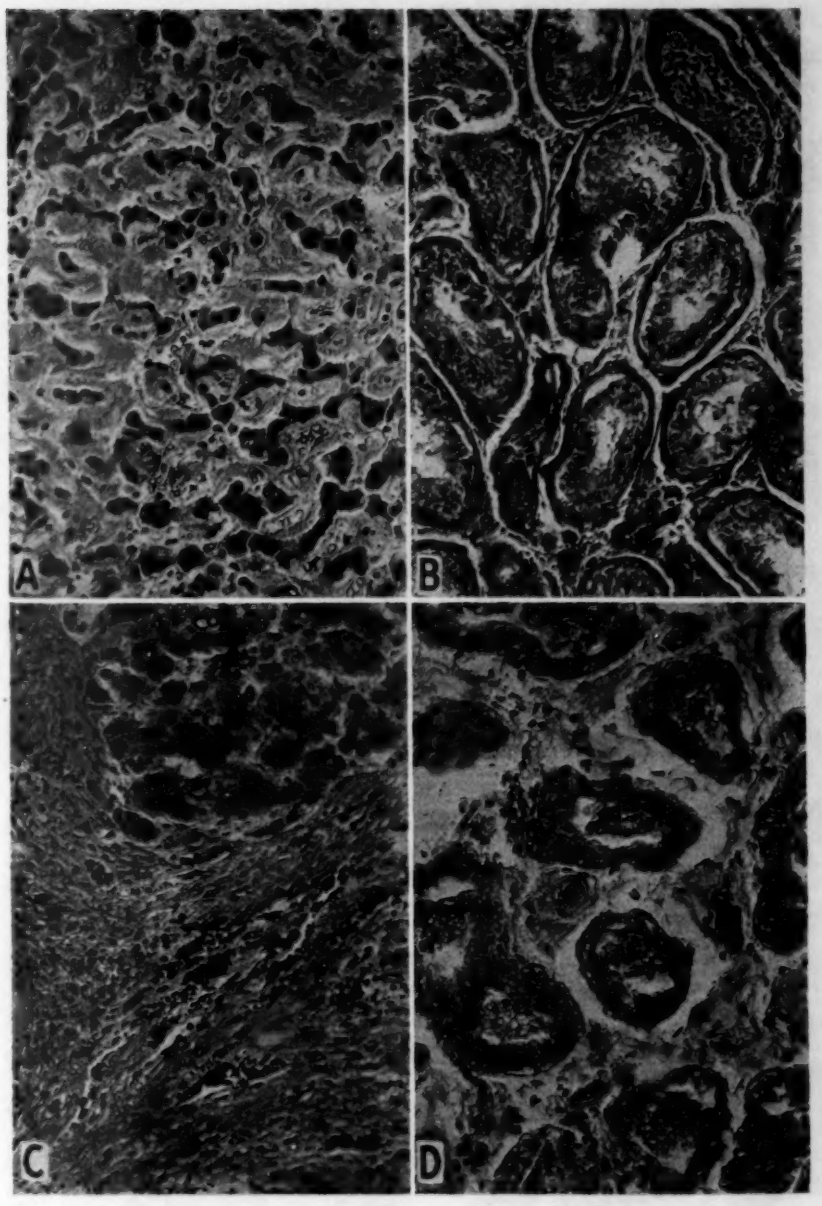
EXPLANATION OF FIGURE 1

A, fibrosis and pseudolobulation in Laennec's cirrhosis in a patient aged 44. There was no history of alcoholism. The albumin-globulin ratio was 1.6 : 4.6. Magnification, × 125.

B, testis from the same patient as *A*. The seminiferous tubules are markedly atrophic. The average diameter of 100 tubules was 118 microns. There is marked thickening of the basement membrane. Sertoli cells are the only tubular cells remaining. There is slight interstitial fibrosis. Magnification, × 150.

C, primary carcinoma of the liver in a man aged 51 with preexisting Laennec's cirrhosis. Connective tissue surrounds an island of liver cells and separates it from the group of tumor cells seen above it. The albumin-globulin ratio was 3.7 : 4.9. Magnification, × 125.

D, testis from the same patient as *C*, showing marked tubular atrophy and almost total cessation of spermatogenesis. The basement membranes are thickened, and there is slight interstitial fibrosis. Magnification, × 75.



EXPLANATION OF FIGURE 2

A, hepatic amyloidosis secondary to carcinoma of the kidney in a patient aged 46. Broad deposits of amyloid separate and compress atrophic cords of liver cells. The albumin-globulin ratio was 3.0 : 3.0. Magnification, × 125.

B, testis from the same patient as *A*. The germinal line of spermatogenésis in the atrophic tubules is frequently reduced to a single layer of Sertoli cells. Magnification, × 75.

C, liver in hemochromatosis in a patient aged 55 with concurrent active pulmonary tuberculosis. Large deposits of iron-containing pigment are seen in the broad fibrous trabeculae, and there are smaller deposits of pigment in the cytoplasm of the hepatic cord cells. The albumin-globulin ratio was 2.1 : 5.8. Magnification, × 125.

D, testis from the same patient as *C*. There is extreme tubular atrophy, with complete fibrosis except for central collections of Sertoli cells. There is no pigmentation but a moderate degree of interstitial fibrosis. Magnification, × 150.

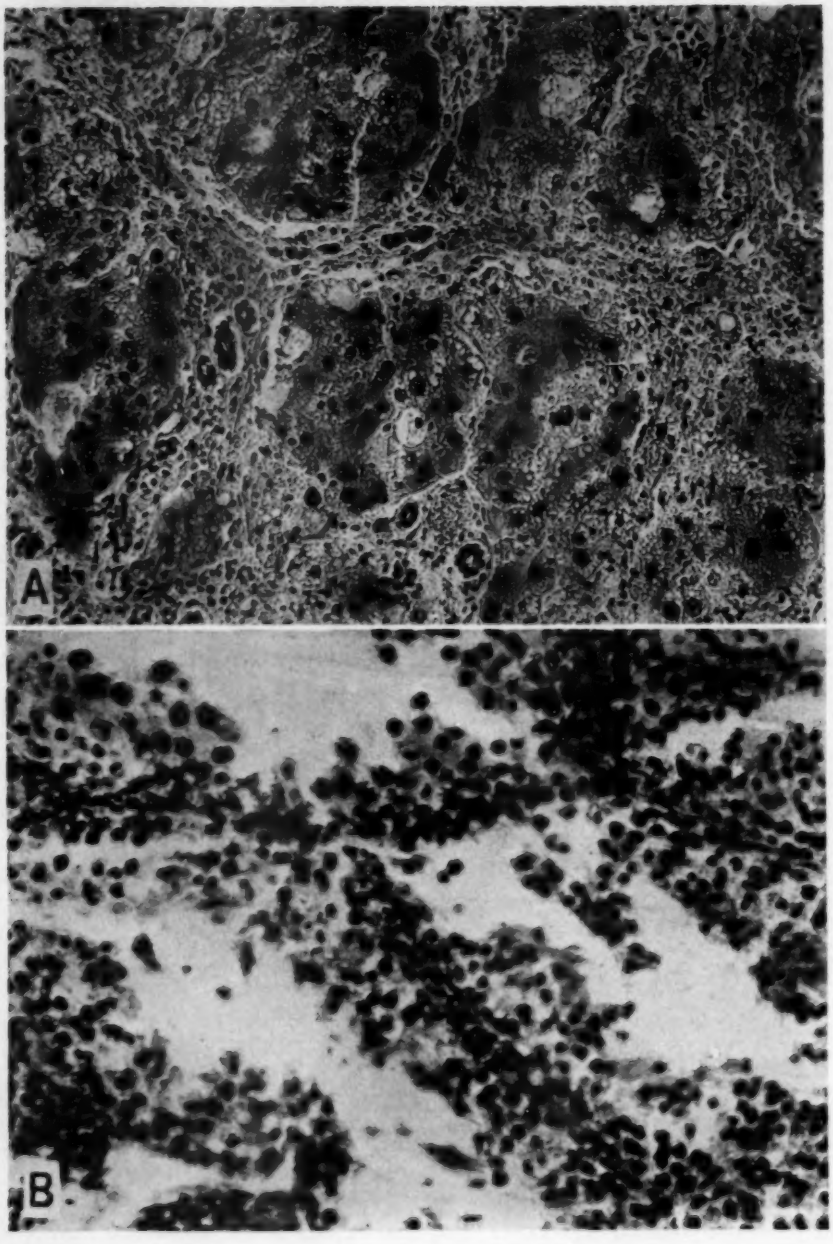


Fig. 3.—*A*, cirrhosis in a rat after seven weeks of exposure to carbon tetrachloride vapors. Ramifying fibrous trabeculae subdivide the hepatic parenchyma into pseudolobules. In the septums there are numerous reduplicated bile ducts and lymphocytes. The remaining islands of liver cells show hypertrophy and fatty degeneration. Magnification, × 125.

B, testis from the same animal as *A*, showing some active spermatogenesis. Spermatogonia, spermatocytes and spermatids are numerous, but there are few adult spermatozoa. Magnification, × 300.

cases of hemochromatosis emphasized sexual hypoplasia in the male patients as an important feature. It manifests itself as impotence and as alterations in secondary sexual characteristics. There is a loss of hair in the axillas and on the chest and trunk, while the pubic hair assumes a characteristic female pattern.

Two cases of hemochromatosis were available for study. The livers in both cases showed extensive fibrosis and pigment deposition. The testes in the first case, that of a man aged 32, showed pronounced thickening of the basement membrane but the germinal epithelium was relatively well preserved. The other case showed extreme tubular atrophy and thickening of the basement membrane but the concurrence of active pulmonary tuberculosis might account for part of these changes (fig. 2 *C* and *D*). In neither case were iron-containing deposits seen in the seminiferous tubules, though minute deposits were seen free as well as within Leydig cells in the interstitial spaces.

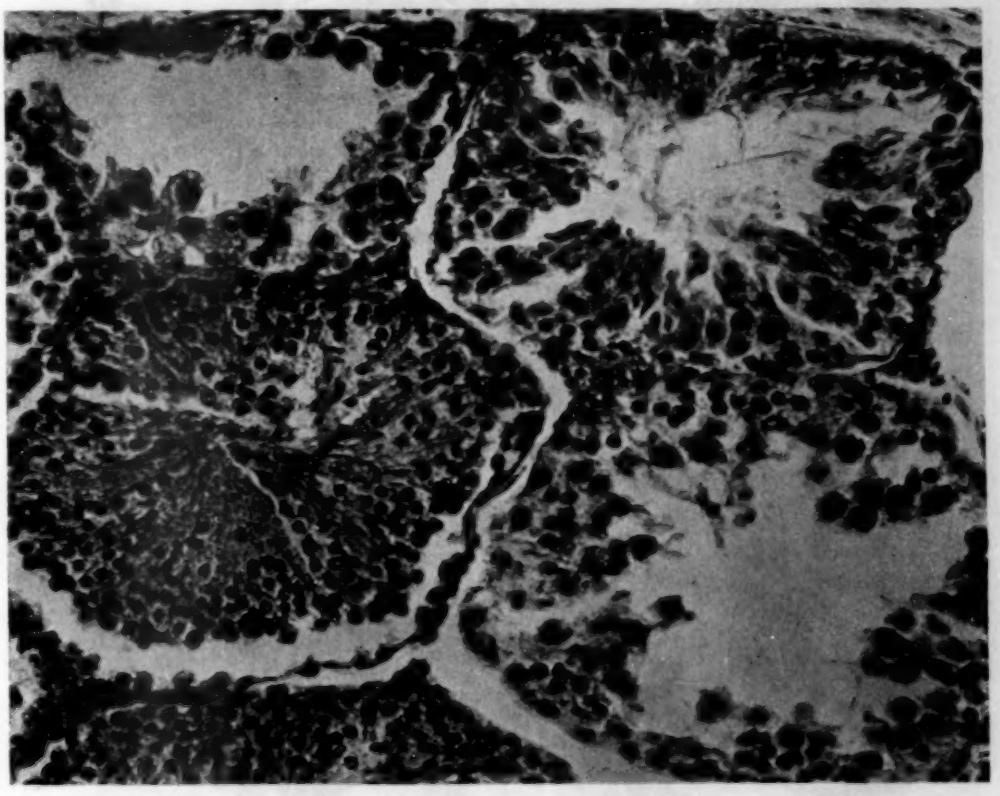


Fig. 4.—Effect on rat testis of a minimum effective dose of estrogen (4 doses of 4 micromilligrams of alpha estradiol dipropionate at five day intervals). The tubule at the lower left is normal, with numerous spermatids. The tubule at the upper right still shows active spermatogenesis, but the tubule beneath it contains no adult spermatozoa. The greatest degree of damage is seen in the atrophic tubule in the upper left corner. These various gradations in tubular damage offer a basis for comparison of the degree of change in the experimental animals in figures 3, 5 and 6. Magnification, × 300.

SUMMARY OF OBSERVATIONS ON HUMAN MATERIAL AND INTRODUCTION TO A REPORT OF ANIMAL EXPERIMENTS

A study of human material indicates, therefore, that hepatic damage is frequently associated with testicular atrophy. If the normal liver may be regarded as an eliminating mechanism protecting the testis against the action of estrogens, testicular atrophy results only when the concentration of circulating estrogens exceeds that which can be inactivated by the liver. If, therefore, one should severely damage the liver by experimental means, the testis should thereafter be unusually susceptible to the deleterious action of administered estrogens, and less administered estrogen should produce more testicular atrophy than is observed in a normal animal with an intact liver.

To investigate this possibility, damage of the liver was produced in rats by inhalation of carbon tetrachloride vapors, and estrogens were then administered to these rats. The common bile duct

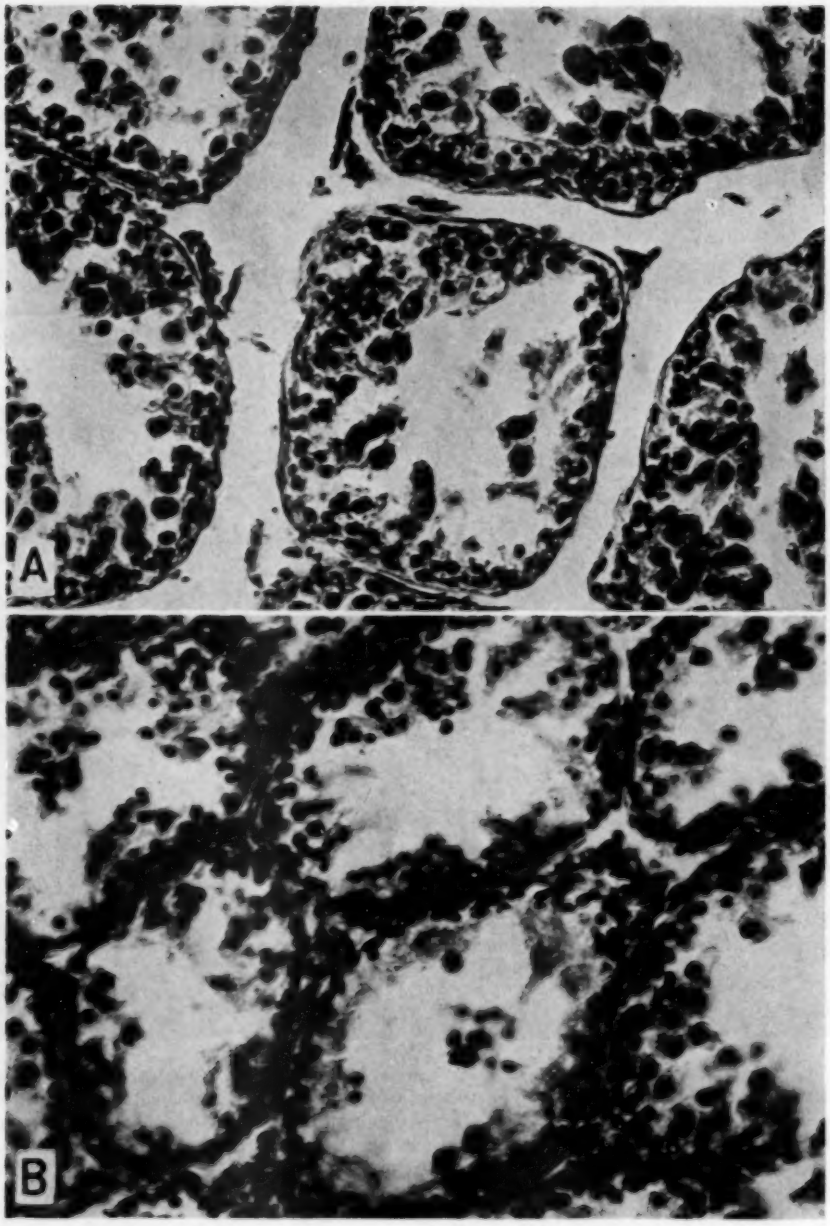


Fig. 5.—*A*, rat testis showing effect produced by a minimum effective dose of estrogen in an animal which had had cirrhosis for seven weeks. Note the irregular thinning of the germinal line of spermatogenesis, the absence of adult spermatozoa and the swollen detached spermatids. Magnification, × 300.

B, effect on the testis of the administration of a minimum effective dose of estrogen to an animal with more severe cirrhosis than *A*. There is a notable diminution in the diameter of the seminiferous tubules, accompanied by still greater destruction of the germinal line of spermatogenesis. There are no adult spermatozoa and only few spermatogonia, spermatocytes and spermatids. Magnification, × 300.

was ligated in another group of animals in order to evaluate the significance of biliary excretion of estrogens in regard to effects on the testis.

EXPERIMENTAL CIRRHOSIS OF THE LIVER AND THE EFFECT OF THE ADMINISTRATION OF AN ESTROGEN

A modification of the method of Forbes [12] was employed in the production of hepatic cirrhosis. Male albino rats, each weighing 115 to 125 Gm., were exposed in a glass-topped air-tight box to carbon tetrachloride vapors for twenty minutes daily six times a week for four weeks. The vaporization was produced with an atomizer introduced through an opening near the top of the chamber. The concentration of vapors was raised to and maintained at a point at which the animals were in a semicomatose state.

At the end of four weeks the animals were killed, and the livers and the testes were examined histologically. The livers from these animals showed advanced cirrhosis, with great increases of connective tissue. The pattern of fibrosis closely simulated that seen in cases of human Laennec's cirrhosis, and stout connective tissue trabeculae traversed and subdivided the hepatic parenchyma into pseudolobules. In addition, lymphocytic infiltration and reduplication of bile ducts were commonly noted among the ramifying fibrous trabeculae. A moderate degree of fatty metamorphosis was frequently seen (fig. 3 *A*).

The testes from these rats with cirrhotic livers either appeared normal or showed only slight diminution of spermatogenesis. This slight degree of damage and lack of fibrosis after a month or seven weeks of exposure to carbon tetrachloride vapors were attributed to the relatively short duration of the cirrhotic state (fig. 3 *B*).

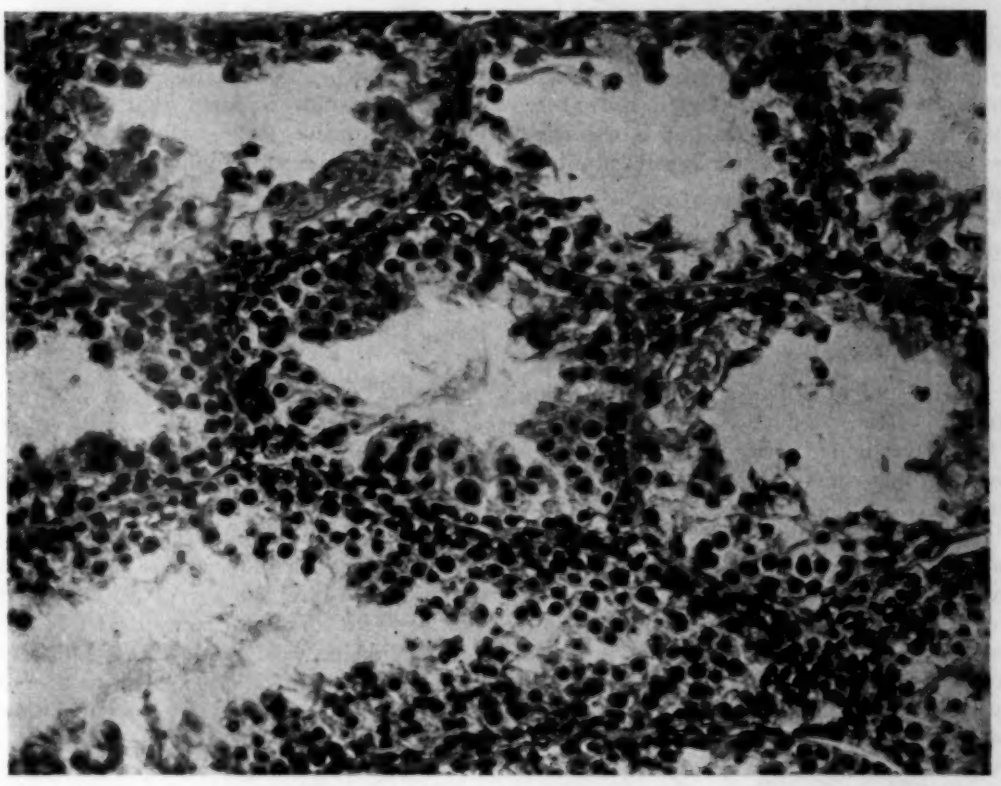


Fig. 6.—Effect of the administration of a minimum effective dose of estrogen to a rat with obstructive jaundice. Spermatogenesis in the central and the lower tubule is active, but elsewhere the germinal line shows paucity of cells and marked diminution in spermatogenesis. Magnification, × 300.

The effect of the administration of an estrogen to rats with cirrhotic livers was then examined. In 42 rats, in assays of various graduated doses of alpha estradiol dipropionate it was found that when 4 micromilligram doses of the preparation were administered at five day intervals for a total of four doses, a constant effect was obtained, namely slight but definite diminution of spermatogenesis (fig. 4). This minimum effective dose was used because it was found that in comparison with a maximum noneffective dose it

12. Forbes, J.: J. Pharmacol. & Exper. Therap. **65**: 287, 1939.

showed a more clearcut enhancement effect when administered to animals with damage of the liver.

Accordingly, 20 rats were exposed to carbon tetrachloride vapors as already described for one month and then were given four doses of 4 micromilligrams of alpha estradiol dipropionate at five day intervals. Their livers and testes were examined at the completion of the course of estrogenic treatment. The livers showed the same extensive cirrhosis that has already been described. It was found that this dosage of the estrogen, which in normal animals had produced only a slight degree of testicular damage, produced striking changes in the testes of these rats with cirrhotic livers. Thus, in 7 of the 12 surviving rats there was marked tubular atrophy with total cessation of spermatogenesis. Moreover, the hepatic cirrhosis was not as extensive in those 5 animals whose testes showed the least damage, so that one may assume that in these animals the better state of hepatic function allowed more inactivation of estrogens and that less testicular atrophy (fig. 5) resulted.

TESTICULAR ATROPHY AND IMPAIRED BILIARY EXCRETION OF ESTROGENS

The significance of impaired biliary excretion of estrogens in the production of an estrogenic effect was investigated by administering alpha estradiol dipropionate to male rats in which the common bile duct had been ligated.

With the rat under ether anesthesia, a ventral abdominal incision was made and through it the common bile duct was cut between two suture ligatures as close as possible to its entrance into the duodenum. The latter precaution assured obstruction of those tributaries which occasionally join the common bile duct near its termination. Of 66 animals operated on, only 19 survived for twenty days. Death in most of them resulted from biliary peritonitis, sepsis and bronchopneumonia. In those animals which survived till the completion of the experiment the proximal segment of the bile duct was distended to a thin-walled sac measuring 1 to 2.5 cm. in diameter. Nine of these animals were controls, but to the other 10, a course of treatment with an estrogen exactly similar to that given to the group with cirrhotic livers had been administered. The livers from both groups showed histologically a striking reduplication of the small biliary radicles, but the parenchymal cells themselves were well preserved.

The effect of obstructive jaundice per se on the testis as noted in 9 animals was negligible. In this group, therefore, inhibition of biliary excretion of endogenous estrogens had little if any significance in the production of testicular atrophy. This conclusion based on these experiments is in accord with the observations on human material presented on foregoing pages. The group receiving alpha estradiol dipropionate, however, showed a moderate degree of testicular atrophy and inhibition of spermatogenesis. The damage, although greater than that seen with the same dose in normal animals, was definitely of less magnitude than that obtained in the group with hepatic cirrhosis (fig. 6).

These results indicate that inactivation of estrogens by liver cells rather than their biliary excretion is the more important mechanism in the natural metabolism of estrogens in males. Thus, an exogenously administered estrogen produced the greatest degree of testicular atrophy when the liver cells themselves were injured, even though in the animals with hepatic cirrhosis bile was still being secreted.

SUMMARY AND CONCLUSIONS

Rigid criteria applied to the testes in 28 cases of Laennec's cirrhosis revealed the presence of significant testicular atrophy in 16 instances, or 57.1 per cent.

The incidence of significant testicular atrophy among persons with cirrhosis of the liver was 90 per cent below the age of 50 years but only 38.9 per cent above the age of 50.

No evidence was found which indicates that chronic alcoholism is associated with testicular degeneration unless it is concurrent with severe hepatic damage.

A study of 34 cases of hepatic failure other than Laennec's cirrhosis suggests that in order to result in atrophy of the testis damage to the liver must be severe, extensive and long standing.

Animal experiments are presented which show that in severe hepatic damage the testis is unusually susceptible to the action of an administered or excess estrogen.

Results of animal experiments point to failure of hepatic inactivation rather than to failure of biliary excretion of estrogens as the mechanism by which testicular atrophy occurs.

SIMILARITY OF THE ACID-FAST PIGMENT CEROID AND OXIDIZED UNSATURATED FAT

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BETHESDA, MD.

In previous reports [1] the lipoid pigment ceroid, occurring in rats fed diets low in choline and in protein, has been described. It occurs as globules in phagocytes in cirrhotic livers and is found less frequently in the lung, the spleen and the lymph nodes. It is pale yellow when unstained and imparts a bronze color to the tissues. In paraffin sections it is acid fast, basophilic, fat positive, iron negative and gram-negative. It is insoluble in water, ordinary fat solvents, dilute acids, dilute alkalis, hydrogen peroxide and chlorine water. It fluoresces in ultraviolet rays.

Ceroid was rather fully characterized in an earlier paper. It was differentiated from hemofuscin, and its possible relationship to the lipoid "pigment" of pneumonia due to aspiration of cod liver oil was suggested.[2] Lung tissue from a human case of pneumonia of this type subsequently studied in this laboratory was found to contain many globules of an insoluble lipoid substance which appeared identical with ceroid.

The nature of the lipoid pigment of pneumonia due to aspiration of oil has been extensively investigated [3] by histologic study, animal experimentation and a few in vitro experiments. This suggested a new approach to the problem of the nature of ceroid. Material for histologic comparison was therefore obtained from several species of animals which had received subcutaneous or intraperitoneal injections of various fats and oils.

Several workers observed that prolonged exposure of certain oils to air or to oxidizing agents results in the formation of insoluble lipoid substances with a strong affinity for carbolfuchsin. These studies have been extended in this laboratory and a substance resembling ceroid has been produced by potassium bichromate oxidation of solidified emulsions of various oils in agar.

This report will present observations on the staining reactions and other properties of the lipoid substances from these various sources and will show that these substances are closely related to one another and to ceroid.

EXPERIMENTS ON ANIMALS

EXPERIMENT 1.—Five hundredths cubic centimeter of cod liver oil U. S. P. XI was injected subcutaneously into each of the four quadrants of the abdomen of a weanling albino rat of the Osborne and Mendel strain. The rat was given the stock diet and on the thirtieth day of the

From the Division of Pathology, National Institute of Health, United States Public Health Service.

1. Lillie, R. D.; Daft, F. S., and Sebrell, W. H., Jr.: Pub. Health Rep. **56**:1255, 1941. Daft, F. S.; Sebrell, W. H., Jr., and Lillie, R. D.: Proc. Soc. Exper. Biol. & Med. **48**:228, 1941. Lowry, J. V.; Daft, F. S.; Sebrell, W. H., Jr.; Ashburn, L. L., and Lillie, R. D.: Pub. Health Rep. **56**:2216, 1941. Lillie, R. D.; Ashburn, L. L.; Sebrell, W. H., Jr.; Daft, F. S., and Lowry, J. V.: ibid. **57**:502, 1942.

2. Endicott, K. M., and Lillie, R. D.: Ceroid, the Dietary Cirrhosis Pigment of Rats, Its Characteristics and Its Differentiation from Hemofuscin, Am. J. Path., to be published.

3. (*a*) Pinkerton, H.: Am. J. Dis. Child. **33**:259, 1927; (*b*) Arch. Path. **5**:380, 1928. (*c*) Graef, I.: ibid. **24**:122, 1937. (*d*) Graef, I.; Kaufmann, W., and Kaplan, L.: ibid. **26**: 914, 1938. (*e*) Graef, I.: ibid. **28**:613, 1939. (*f*) Hass, G. M.: ibid. **26**:956, 1938; (*g*) **26**: 1183, 1938; (*h*) **26**:1196, 1938; (*i*) **27**:15, 1939; (*j*) **28**:177, 1939.

experiment was killed and examined. In the subcutaneous tissue at the sites of the injections were found small cysts filled with orange floccular material and surrounded by moderately firm tissue having an orange yellow color. Histologic examination of material fixed in 4 per cent solution of formaldehyde and embedded in paraffin revealed small cysts containing a thin peripheral rim of acid-fast basophilic material and lined by foreign body giant cells and macrophages. In the moderately dense, lymphocyte-infiltrated tissue surrounding the cysts were macrophages and foreign body giant cells containing acid-fast basophilic globules.

EXPERIMENT 2.—Twelve adult albino rats fed the stock diet were used for this experiment. Three rats received 5 cc. of cod liver oil intraperitoneally, 3 received 5 cc. of crude linseed oil, 3 received 5 cc. of beef lard and 3 received 5 cc. of a commercial hydrogenated cottonseed oil used for cooking (Crisco).

Of the rats given cod liver oil, 2 were found dead on the tenth day. The third rat was killed on the forty-eighth day. All three showed yellow floccular material in the peritoneal cavity. Histologic study of tissue fixed in solution of formaldehyde and embedded in paraffin revealed a proliferative reaction of the peritoneum, most marked on the diaphragm and near the spleen. In the loose fibroblast network were cystic spaces with peripheral rims of acid-fast basophilic material. Of the 3 rats given linseed oil, 1 was killed on the fourteenth, 1 on the thirtieth and 1 on the forty-eighth day. All 3 showed yellowish floccules on the peritoneal surfaces and on microscopic examination revealed many small foreign body granulomatous foci in which were scattered giant cells containing acid-fast basophilic droplets. The rats given the hydrogenated cottonseed oil and those given lard were killed and examined at similar intervals. All showed foreign body reactions, but no acid-fast basophilic material could be found.

Similarly oils prepared from mouse and rat livers and rat body fat gave foreign body reactions but no acid-fast basophilic material.

EXPERIMENT 3.—Cod liver oil U. S. P. XI was hydrogenated in a pressure bomb at an initial pressure of 2,100 pounds per square inch (148 Kg. per square centimeter) at 100 C. in the presence of Raney nickel catalyst and with constant agitation in a mechanical shaking device. (Dr. E. M. Fry carried out the hydrogenation of the cod liver oil.) The product was filtered to remove the catalyst. It was a white waxy solid at room temperature (melting point 60 to 65 C.) and when melted was clear and colorless. Four cubic centimeters of this hydrogenated cod liver oil was injected into the peritoneal cavity of each of 2 weanling albino rats; 0.5 cc. was injected into the subcutaneous tissue of each of 2 weanling albino rats. The rats were fed the stock diet. At the end of one month the rats were killed and examined. The solidified oil in the peritoneal cavity was found completely walled off by adhesion of viscera. Microscopically, the wall consisted of a thick inner zone of necrotic neutrophil-infiltrated granulation tissue and an outer zone of densely cellular fibrous tissue. In the subcutaneous tissue were many cysts lined by macrophages and foreign body giant cells. There were marked proliferation of fibroblasts and exudation of neutrophilic leukocytes. No acid-fast basophilic lipoid substance was found.

IN VITRO EXPERIMENTS

Group 1.—Four emulsions, each containing 5 per cent cod liver oil, were prepared by agitation in the Waring blender. One emulsion was made from hot 2 per cent agar, one from hot 25 per cent gelatin, one from 5 per cent blood plasma (dry weight) and one from 25 per cent egg albumin (dry weight). The emulsions were poured into test tubes and were coagulated by immersion in boiling water (plasma and albumin) or by cooling (agar and gelatin). Plugs of each coagulated emulsion were then dislodged into 5 per cent aqueous potassium bichromate, 5 per cent aqueous potassium permanganate, solution of hydrogen peroxide U. S. P. and distilled water. At intervals of one week, slices were cut from the plugs and from untreated controls and were dehydrated in acetone, cleared in gasoline, embedded in paraffin, sectioned, mounted on slides, passed through xylene and alcohol, stained with eosin–polychrome methylene blue and with steaming carbolfuchsin, and studied microscopically. Untreated controls showed a uniform appearance throughout. The matrix of agar, gelatin, plasma or albumin showed numerous vacuoles representing spaces from which the cod liver oil had been removed. In those placed in potassium bichromate acid-fast basophilic globules developed about the periphery of the plug. This acid-fast peripheral zone was present in one week and became progressively wider. It was noted that the change of cod liver oil from its natural state to the insoluble acid-fast material took place first at the surface of each individual droplet so that in some droplets only a thin rim at the periphery of the droplet had been altered and persisted in the paraffin sections as an acid-fast basophilic rim. This same

sequence of events has been noted in the droplets of fat in cirrhotic livers of choline-deficient rats. Plugs treated in potassium permanganate were rapidly covered with and impregnated by a black precipitate of manganese dioxide and became friable, difficult to section and unsatisfactory for microscopic study. Plugs treated in distilled water and in hydrogen peroxide showed no insoluble lipoid material at the end of six weeks.

Group 2.—Three emulsions in 2 per cent agar were prepared by using 10 per cent crude linseed oil, 10 per cent hydrogenated cottonseed oil (Crisco) and 10 per cent beef lard, respectively. The emulsions were coagulated by cooling in staining dishes and were cut into 1 by 1 by 3 cm. blocks. This method was adopted to obviate the difficulty of dislodging the plugs from test tubes. Blocks were placed in 5 per cent potassium bichromate, and at intervals of one, two, three, four and eight weeks slices were removed and prepared for study as before. The hydrogenated cottonseed oil emulsion and the lard emulsion remained negative throughout. The linseed oil emulsion showed acid-fast peripheral droplets in one week. In two weeks the acid-fast droplets were basophilic. Thereafter the peripheral zone of altered oil droplets became progressively wider.

Group 3.—An emulsion of 10 per cent hydrogenated cod liver oil in 2 per cent agar was prepared and treated as in group 2. No acid-fast or basophilic droplets were found at any time.

STAINING REACTIONS AND OTHER PROPERTIES

The preliminary studies just described revealed acid-fast basophilic lipoid material in those animals given injections of cod liver oil and linseed oil and in the oxidized emulsions containing cod liver oil and linseed oil. Representative material was selected from each for further studies. Paraffin sections of formaldehyde-fixed lung from a human case of pneumonia due to aspiration of oil were included for comparison. The patient was a hydrocephalic infant who died after prolonged hospitalization during which he was given cod liver oil daily by mouth. The grossly consolidated left lung showed microscopically a diffuse fibrinopurulent alveolar exudate with scattered foci containing globules of acid-fast basophilic lipoid material in macrophages, in multinucleated giant cells and lying free in alveoli.

All stains and solubility tests were carried out with paraffin sections so that the ordinary tissue lipids had been removed. Sections from the various sources were tested simultaneously. The globules and peripheral rims of insoluble lipoid material varied somewhat in intensity of staining both from globule to globule in the same section and in sections from different sources, but all showed the following characteristics in common: They were orange red with sudan IV, blue to purple with nile blue sulfate and black with osmic acid. They were acid fast, stained slowly in the basic aniline dyes, were gram negative, were iron negative (hot hydrochloric acid–potassium ferrocyanide test of Perles) and were unstained in hemalum and eosin, in which they showed a pale yellow color. They all stained gray to black in Weil's modification of the Weigert stain for myelin. All reduced diamino silver carbonate solution (Foot's), taking a brown to black color. None were dissolved after ninety-six hours at room temperature in absolute alcohol, acetone, chloroform, xylene, solution of hydrogen peroxide U. S. P., 1 per cent aqueous hydrochloric acid, 1 per cent aqueous sodium carbonate or distilled water. When observed in ultraviolet rays all showed a greenish yellow fluorescence with the exception of the emulsions oxidized with potassium bichromate, which contained no fluorescent material. Ceroid loses its fluorescence but retains its acid fastness and basophilia when the sections are placed for one hour in 5 per cent potassium bichromate.

COMMENT

The staining reactions and other properties enumerated are identical with those of ceroid as described in a previous report. Naturally, staining reactions and solubilities cannot be accepted as absolute proof, but the remarkable resemblance lends strong support to the presumption that these substances are closely related if not identical.

The nature of the acid-fast material in lipid pneumonia has been investigated by a number of workers. Pinkerton,[3a, b] injected various oils intratracheally in rabbits and puppies. He found that cod liver oil after a prolonged stay in the lungs became acid fast. He stated that fixation in solution of formaldehyde or in Zenker's solution rendered cod liver oil insoluble in ordinary fat solvents. He bubbled air through cod liver oil and obtained a thick gummy acid-fast substance insoluble in

ether and chloroform. He suggested that the acid fastness and insolubility may be due to hydrolysis and oxidation.

Graef and his associates [3c,e] reported a number of human cases of lipid pneumonia and presented staining methods for the identification of the aspirated lipoid substance. They injected eighteen different oils intratracheally in rabbits and monkeys and found acid-fast membranes about injected sperm oil, chaulmoogra oil, cod liver oil, halibut liver oil, tung oil and linseed oil and about the ethyl esters of cod liver oil and linseed oil. They reported that the membranes persisted in paraffin sections, where they were acid fast and fat positive. They expressed the belief that the acid-fast membrane is a lipoprotein.

Hass [3f, j] investigated the subject extensively. He injected lipoid substances into the abdominal subcutaneous tissues or spleens of young guinea pigs. After investigating a large number of crude and purified fats, oils, fatty acids and esters of fatty acids, he concluded that long chain fatty acids having several double bonds or esters of such acids are necessary for the production of the acid-fast material. He supported the theory that the mechanism involved is an oxidation of the unsaturated fatty acids at the double bonds with the formation of a peroxide and the union of a number of molecules by polymerization. He suggested that a lipoprotein may be formed at the interface of the lipoid and aqueous phases.

The experimental results reported here are in agreement with the experience of other workers. An acid-fast basophilic insoluble lipoid substance was produced by parenteral administration of cod liver oil and linseed oil. It was not produced by injection of hydrogenated cod liver oil, beef lard or hydrogenated cottonseed oil, all of which contain little or no highly unsaturated long chain fatty acids.

Cod liver oil and linseed oil yielded acid-fast basophilic insoluble material after prolonged treatment with potassium bichromate in vitro. Hydrogenated cod liver oil, hydrogenated cottonseed oil and beef lard failed to do so. The changes which occur must be, at least in part, oxidation. Furthermore, since the reaction occurs only with lipoid substances containing rather large amounts of such acids as linolenic or linoleic or their esters, it appears probable that these acids are the substances which are oxidized. The substance produced by this oxidation cannot be a lipoprotein since in an agar emulsion of cod liver oil there is practically no protein. It seems likely, therefore, that the animal material is not a lipoprotein.

The exact point at which oxidation occurs and the chemical structure of the intermediate and the end products remain in doubt. Other workers have pointed out that the process involved here may be similar to that which occurs in the preparation of "blown" oils. An interesting discussion of the chemistry of the blown oils is given by Glimm.[4] Several different chemists have reached the conclusion that there is an oxidation of the fatty acids at the double bonds with the formation of unstable peroxides which are subsequently polymerized, possibly after passing through a ketoalcohol stage. Perhaps a similar reaction is responsible for the formation of ceroid.

Choline appears to be intimately connected with the formation of ceroid since ceroid has been reported to occur spontaneously only in rats fed choline-deficient diets. However, diets apparently adequate in choline do not prevent the formation of ceroid-like material in rats, mice, dogs, rabbits, guinea pigs, monkeys or man when lipoid substances containing highly unsaturated long chain fatty acids or esters are introduced artificially into the lungs, the subcutaneous tissue, the peritoneal cavity or the spleen. It remains to be found whether choline has some

4. Glimm, E.: Fette u. Seifen **46**:348, 1939.

specific effect on the metabolism of unsaturated fatty acids or whether it acts simply as a fat mobilizer and prevents the accumulation of large amounts of fat in the liver where the fat may undergo this peculiar alteration either within a living liver cell or in the interstices following death and rupture of the cell.

SUMMARY

An insoluble acid-fast basophilic lipoid substance has been produced in rats by injection of cod liver oil and linseed oil. A similar substance has been produced by prolonged oxidation of solidified agar emulsions of cod liver oil and linseed oil with potassium bichromate. A similar substance has been encountered in a human case of pneumonia due to aspiration of cod liver oil. All of these substances have been studied with respect to their behavior toward stains and solvents. They have been found to bear a remarkable resemblance to one another and to ceroid, the pigment of rats with dietary cirrhosis. The mechanism involved in the production of the lipoid substances is still in question.

UNUSUAL CARDIAC LESIONS ASSOCIATED WITH CHRONIC MULTIPLE RHEUMATOID ARTHRITIS

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In a previous study [1] on cardiac lesions associated with rheumatoid arthritis it was noted that in 1 instance (case 6) cardiac lesions were present which were strikingly similar to the subcutaneous nodules of chronic multiple rheumatoid arthritis. Since that report, another case in which there were lesions of the same type has come to our attention. The similarity of the cardiac lesions in these 2 cases to the subcutaneous nodules of rheumatoid arthritis has led us to the question whether we were dealing with a form of heart disease peculiar to patients suffering from rheumatoid arthritis.

REPORT OF CASES

CASE 1.—The patient was a woman aged 59 years at the time of death. In 1913, when she was aged 41 years, pain and swelling in the joints of the hands and the feet had developed. Gradually the knees, the shoulders, the elbows and the wrists became involved. The patient was observed first at the Mayo Clinic in 1914 and again in 1918. On the occasion of the second visit she was badly crippled. The right knee was greatly swollen, the fingers were deformed and the wrists, the shoulders and the right hip were stiff and painful on motion. The concentration of hemoglobin was 40 per cent; the erythrocytes numbered 3,360,000 and the leukocytes 4,600 in each cubic millimeter of blood. Roentgenograms of the right knee showed no abnormality, but roentgenograms of the right hand revealed evidence of rheumatoid arthritis.

In 1925, at the age of 53 years, the patient returned again for observation. She reported that the arthritis had been steadily progressive; all of the large joints had become involved, and even the temporomandibular joints were stiff and painful on motion. For seven years she had been able to be around only in a wheel chair. For about six months she had been having smothering sensations and difficulty in breathing. She could not lie down without experiencing shortness of breath. The knees were held at right angles by flexion contractures. Laboratory examination in 1925 showed a faint trace of albumin in the urine. The concentration of hemoglobin was 70 per cent; the erythrocytes numbered 4,010,000 and the leukocytes 4,600 in each cubic millimeter of blood. Roentgenograms of the knees and hands showed marked destructive arthritis and ankylosis.

After this examination the patient disappeared from the observation of the clinic; she died six years later. No information could be obtained concerning the nature of her final illness.

Necropsy.—There were flexion deformities of the fingers, the hips and the knees and severe edema of both lower extremities. The left pleural cavity was partially obliterated by fibrous and fibrinous adhesions. It contained approximately 1,000 cc. of bloody fluid, and the right pleural cavity contained 500 cc. of amber-colored fluid. There were a few fibrous adhesions at the apex of the right lung. The pericardial sac measured 16 cm. transversely and contained approximately 200 cc. of blood-tinged fluid.

The heart weighed 547 Gm. The parietal and visceral layers of the pericardium were covered by a heavy fibrinous exudate. The tricuspid and pulmonary valves were not remarkable grossly. The leaflets of the mitral valve were moderately thickened and stiffened. The thickening was most pronounced near the valve ring. There were small raised nodules along the line of closure and above it on the auricular surface. The chordae tendineae were shortened and thickened, and moderate mitral stenosis was present. The endocardium of the left auricle was thickened and roughened. The endocardium of the papillary muscles of the left ventricle also was thickened.

The aortic leaflets were moderately thickened and stiffened, and the edges were rounded. There was mild stenosis. The intimal surface of the ascending aorta did not appear to be remarkable grossly. The sclerosis of the coronary arteries was mild.

The lungs were not remarkable except for moderate atelectasis of both lower lobes and a caseocalcareous nodule, 2 mm. in diameter, in the apex of the upper lobe of the left lung.

The spleen was enlarged and weighed 315 Gm. but was otherwise not remarkable.

The liver weighed 1,440 Gm. In the right lobe there was a subcapsular calcified nodule which was 2 mm. in diameter.

The cervix was stenotic, and the endometrial cavity contained approximately 20 cc. of thick yellow pus. The uterine tubes and the ovaries were not remarkable.

Histologic Examination.—Mitral Valve: The mitral valve and ring were the site of a chronic inflammatory process. The predominant structural unit of this inflammatory process was a granulomatous body which was roughly spherical in the valve ring but elongated and bandlike in the leaflet. These structures possessed three well demarcated zones (fig. 1). The central zone generally consisted of acidophilic, apparently necrotic tissue. Necrosis was not always complete, as sections stained by the Gömöri[2] method for reticulum frequently revealed an intact reticular and collagenous framework. The central portion generally was acellular but occasionally contained numbers of degenerating leukocytes. In some lesions calcification had occurred. Immediately adjacent to and sharply demarcated from the central necrotic portion was a zone of large, elongated, radially directed cells. These cells had large pale-staining nuclei and faintly basophilic cytoplasm. Their borders were frequently indistinct. The cells appeared to be proliferating extensively and were

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* Work done while Dr. Rosenberg was a Fellow in Medicine at the Mayo Foundation.

From the Section on Pathologic Anatomy, Mayo Clinic (Dr. Baggenstoss).

1. Baggenstoss, A. H., and Rosenberg, E. F.: Arch. Int. Med. **67**:241, 1941.

2. Gömöri, G.: Am. J. Path. **13**:993, 1937.

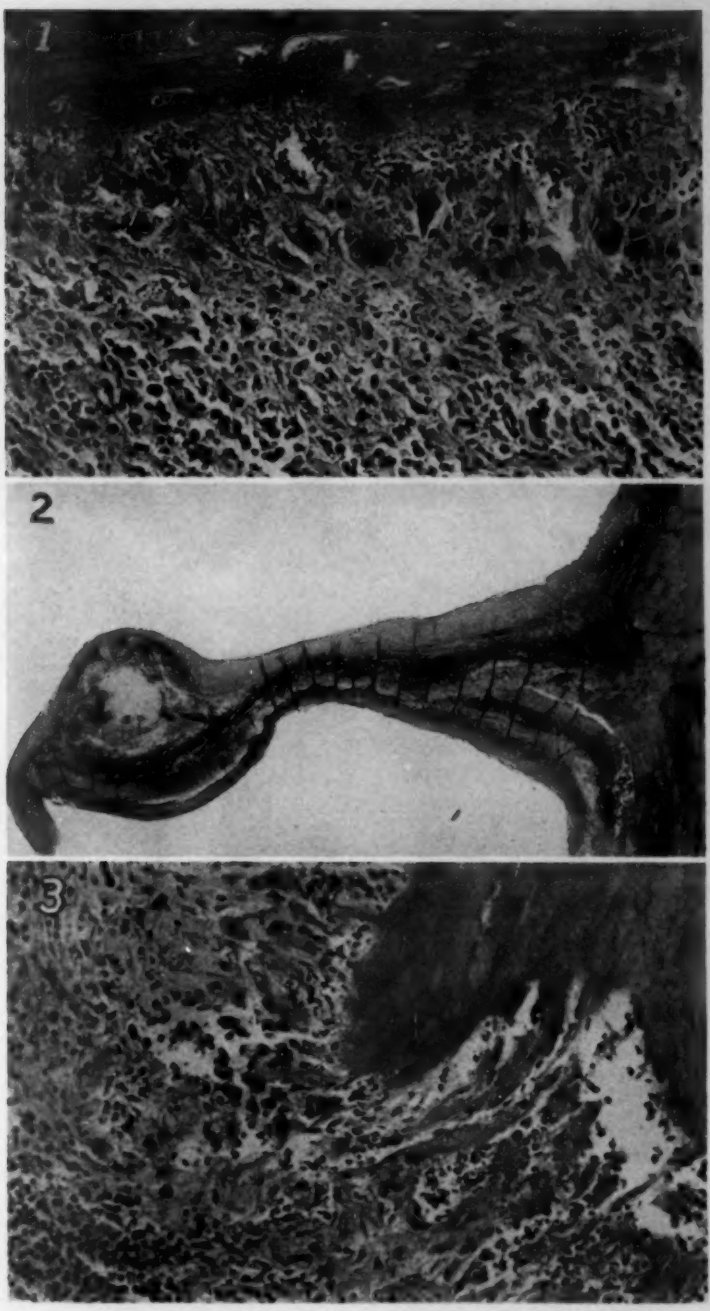


Fig. 1 (case 1).—Nodule in the mitral valve. The zones of necrosis, the large palisaded cells and the peripheral inflammatory reaction are shown (hematoxylin and eosin; × 195).

Fig. 2 (case 1).—Aortic valve showing extensive nodular and bandlike lesions in the leaflet and the valve ring (hematoxylin and eosin; × 8).

Fig. 3 (case 1).—Nodule in the aortic valve (hematoxylin and eosin; × 100).

frequently multinucleated (fig. 1). They were often associated with collagenous and reticular fibrils, and consequently they were interpreted as fibroblasts and undifferentiated mesenchymal or reticular cells. The most striking features about them were their large size and their radial or palisaded arrangement. Peripheral to the intermediate zone of palisaded cells was a broad and imperfectly delineated zone of inflammatory reaction. The chief constituents of this zone were collagenous connective tissue, blood vessels, fibroblasts, macrophages, lymphocytes and plasma cells. Of the cellular elements, plasma cells and lymphocytes were the most numerous and generally were found in the interstices of the collagenous connective tissue framework. Sections appropriately stained for Treponema pallidum and Mycobacterium tuberculosis failed to reveal either of these organisms. In addition to the granulomatous structures there were irregular regions of fibrosis and collections of lymphocytes and plasma cells. Vascularization by capillaries and large vessels was prominent. The larger vessels were often thickened, and occasionally the lumens were entirely obliterated.

Aortic and other valves: In the aortic valve and ring there were lesions similar to those described in the mitral valve (figs. 2 and 3). The lesions of the valve ring and the annulus fibrosus were continuous with those of the pericardium and the aorta. The tricuspid valve was not abnormal, but there was a small focus of acute endocarditis on one of the leaflets of the pulmonary valve.

Pericardium: The visceral and parietal layers were the sites of the same granulomatous inflammatory process described in the mitral and aortic valves. In some of the lesions the necrotic central portions consisted largely of degenerating leukocytes, and in others the central portion had apparently become liquefied and absorbed, since the intermediate zone of cells lined a cystlike space. On the surface of the pericardium in some sections the large fibroblastic and reticular cells bordered a layer of fibrinous exudate. Some of the lesions of the pericardium were small and were similar to myocardial Aschoff bodies (fig. 4).

Aorta: The adventitia of the aorta had lesions similar to and continuous with those of the pericardium. The media revealed similar lesions. In some regions

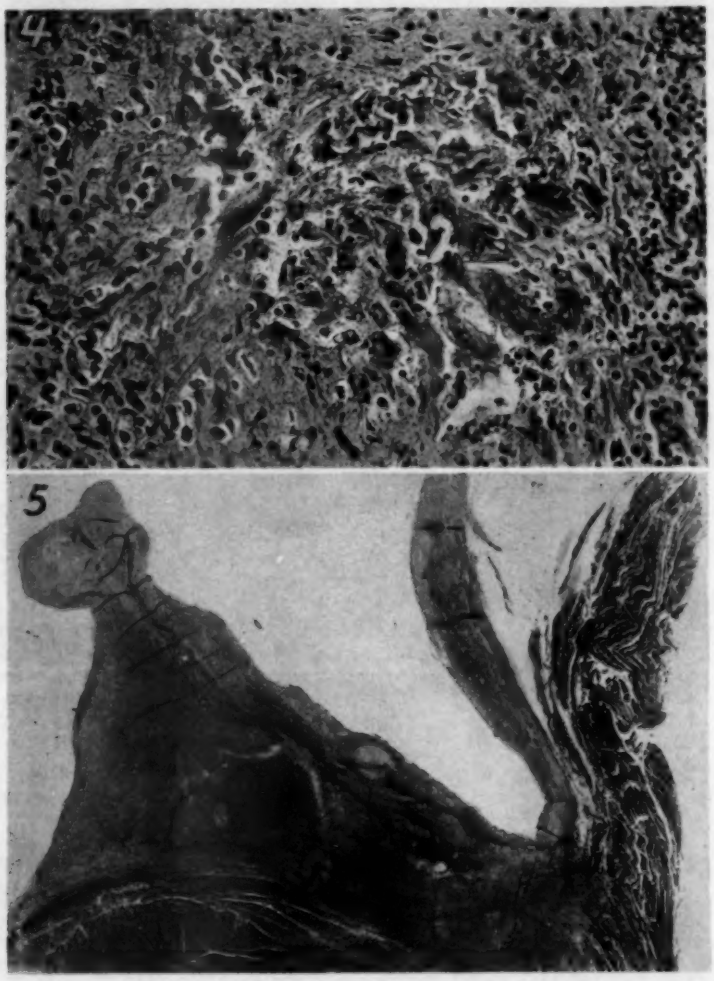


Fig. 4 (case 1).—Small cellular nodule from the pericardium (hematoxylin and eosin; × 200).

Fig. 5 (case 2).—Aortic valve showing an extensive lesion in the leaflet and the ring (hematoxylin and eosin; × 8).

of the media the necrosis appeared to be more recent and was associated with collections of degenerating polymorphonuclear leukocytes without a well developed intermediate zone of the large fibroblasts and reticular cells. In some of the lesions of the media the predominating cellular elements were plasma cells and lymphocytes. The arterioles of the vasa vasorum had greatly thickened walls. Perivascular collections of lymphocytes and plasma cells were common.

Myocardium: Focal collections of lymphocytes, plasma cells and reticular cells were scattered throughout the interventricular septum. These collections were usually in relation to blood vessels but occasionally lay between muscle bundles at a distance from any discernible vessels. Occasionally these collections had the appearance of Aschoff bodies.

Lungs: There was moderate chronic passive congestion together with pulmonary arteriosclerosis and arteriolosclerosis. There was also mild alveolar emphysema. The caseocalcareous nodule of the upper lobe of the left lung revealed evidence of mildly active chronic tuberculosis.

Spleen: There were moderate chronic passive congestion and arteriosclerosis. There was mild hyperplasia of the reticular cells of the pulp.

Liver: Moderate chronic passive congestion with mild atrophy of the hepatic cells in the region of the central veins was noted. There was also moderate arteriosclerosis.

Kidneys: Moderate glomerulitis (endothelial proliferation, grade 2 on the basis of 4 as the most severe) was present in the capillaries of the glomerular tufts. There was mild fatty change in the epithelial cells of some of the proximal convoluted tubules. There was mild arteriosclerosis.

Uterus: There were collections of polymorphonuclear cells, plasma cells and macrophages in the endometrium.

Other Organs: Evidences of focal arteritis were noted in the arteries of the broad ligament and in those of the pancreas. The histologic sections of other organs were not remarkable.

Anatomic Diagnosis.—The anatomic diagnosis was as follows: chronic rheumatic (mitral and aortic) endocarditis, myocarditis, pericarditis and aortitis; chronic multiple rheumatoid arthritis; hydrothorax (left, 1,000 cc.; right, 500 cc.); healing tuberculosis of the left lung; pyometria; glomerulitis of the kidneys; chronic passive congestion of the lungs, the liver and the spleen; generalized and pulmonary arteriolosclerosis.

CASE 2.—The patient was a woman aged 37 years when she first presented herself at the clinic in February 1928. Painful swelling of the metatarsal joints had developed at the age of 27 years. There had been further trouble of this nature from time to time ever since. Approximately six months before registration the patient had begun to suffer from pains in the shoulders, the back and the arms. Three weeks later an increase of pain and swelling appeared in the metatarsal regions of both feet and in the fingers of both hands.

Physical examination showed swelling and tenderness of the metatarsal joints. The joints of the hands were painful but not swollen. Urinalysis showed a trace of albumin (1+). The concentration of hemoglobin was 67 per cent (Dare); the erythrocytes numbered 4,060,000 and the leukocytes 7,500 in each cubic millimeter of blood. Roentgenograms of the ankles and feet showed no abnormality. The Wassermann reaction for syphilis was negative.

A diagnosis of mild chronic multiple arthritis was made. The patient returned in October 1931. On this occasion the range of motion of the shoulders was limited to a third of the normal, and these motions were painful. The elbows were held in 20 degrees of flexion. The motions of the fingers were limited and painful. The ankles and the metatarsal regions were swollen and tender. The knees were swollen but painless.

Roentgenograms of the feet showed evidences of marked atrophy of the bones, while those of the knees revealed evidence of periarticular arthritis.

Course.—On Dec. 7, 1931, in the hope of improving the patient's arthritis, bilateral lumbar sympathetic ganglionectomy and resection of sympathetic trunks were performed. The patient recovered uneventfully from this procedure. She seemed to have less pain in affected joints while resting but continued to have much pain in the joints of the lower extremities when she tried to walk. She was dismissed March 16, 1932. She did not return to the clinic, and her death was reported to us in January 1941.

Necropsy.—Necropsy was partially performed elsewhere, and the heart, a small specimen of liver and the phalangeal joints of the index finger of the left hand were sent to us for study.

The heart weighed 217 Gm. There were fibrous adhesions attached to the epicardium. The leaflets of the aortic valve were greatly thickened and appeared to be somewhat shortened. The free edges of the leaflets were rounded and thickened. The thickening was most marked at the base of the leaflet, and the normal subvalvular angle was partially obliterated by fibrous connective tissue (fig. 5). The sinuses of Valsalva and the aorta were not remarkable. The coronary orifices were not compromised. The mitral valve did not appear remarkable except for slight thickening at the base of the leaflet. The chordae tendineae and the papillary muscles appeared normal. The tricuspid and pulmonary valves appeared normal grossly.

The specimen of liver did not appear remarkable grossly.

As to the joints of the index finger, there was fibrous ankylosis of the distal interphalangeal joint. On sagittal section there was no evidence of any articular cartilage and the joint space was filled with fibrous connective tissue.

Histologic Examination.—Aortic Valve: The leaflets were greatly thickened by fibrous connective tissue. The thickening was most prominent on the ventricular surface and had led to obliteration of the normal subvalvular angle (fig. 5). In the leaflet, as well as in the valve ring, there were revealed large and small lesions entirely similar to those described in the valves in case 1 (fig. 6). The central region of necrosis, the intermediate zone of large pallisaded cells and the peripheral inflammatory reaction were all present. By the use of silver impregnation, intact reticular and collagen fibrils could also be demonstrated in the central portions of the nodules. The histologic appearance of the nodules was similar whether the nodules were large or small. Sections stained for T. pallidum and M. tuberculosis failed to reveal these organisms.

Mitral Valve: There was slight thickening of the leaflet by hyalinized connective tissue, which was well vascularized. In the valve ring there were nodules similar to those described for the aortic valve. Partial calcification of the central portion had occurred in some of them (fig. 7 *a*). Histiocytes, fibroblasts and myo-

cardial reticulocytes (Anitschkow myocytes) were observed at the edge of the necrotic region (fig. 7 *b*).

Interventricular Septum: No typical Aschoff bodies were found. There were numerous perivascular ("onion skin") scars, however. Such scars were considered by Klinge [3] to be the residual scars of Aschoff nodules (fig. 8). A number of the arterioles possessed raised nodules on the intimal surface, which were interpreted as focal proliferations of the endothelial cells. Examination of the ascending aorta did not reveal any significant lesions.

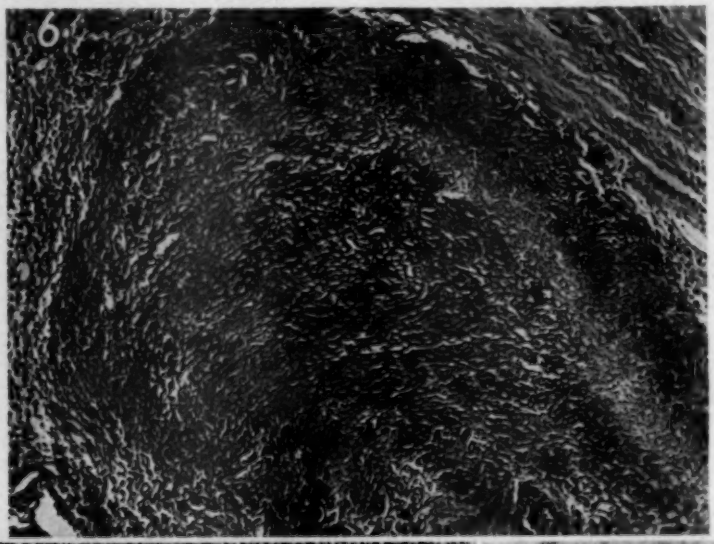
Liver: There was evidence of mild to moderate chronic passive congestion and of moderate atrophy of the hepatic cells about the central veins of the lobules. There was moderate fatty change in these cells.

3. Klinge, F.: Ergebn. d. allg. Path. u. path. Anat. **27**:154, 1933.

COMMENT

The fundamental nature of the cardiac lesions in these 2 cases was difficult to determine. Three possibilities presented themselves: (1) The lesions represented some granulomatous disease unrelated to either rheumatoid arthritis or rheumatic fever; (2) the lesions were peculiar to and specific for rheumatoid arthritis; (3) the lesions were a manifestation of rheumatic heart disease.

The possibility that these lesions might be a manifestation of tuberculosis was considered but was ruled out by the considerations that active or progressive tuberculosis was not present else-



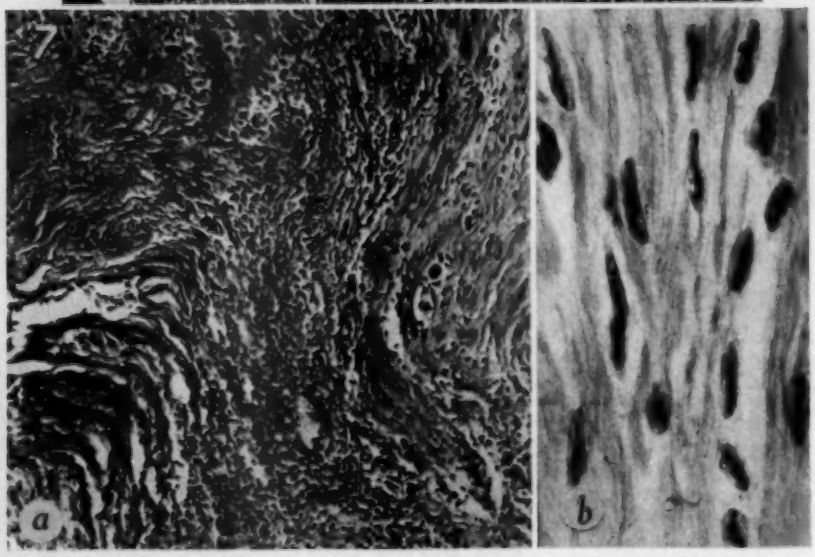


Fig. 6 (case 2).—Lesion in the aortic valve (hematoxylin and eosin; × 80).

Fig. 7 (case 2).—(*a*) The edges of two lesions are shown; calcification has occurred in the one on the left (hematoxylin and eosin; × 100). (*b*) Myocardial reticulocytes (Anitschkow myocytes) (hematoxylin and eosin; × 650.

where in either of the cases, that tuberculosis is rarely observed in the valves of the heart, that the histologic picture was not typical of tuberculosis and that M. tuberculosis could not be found in the lesions. The possibility that we were dealing with a manifestation of syphilis was also considered. This diagnosis was dismissed in spite of unsatisfactory serologic evidence because of the following facts: Signs of syphilis were absent elsewhere in the body; lesions were present in the pericardium and mitral valves, which rarely, if ever, are involved in syphilis; the histologic appearance was not typical of syphilitic lesions; T. pallidum could not be found in appropriately stained sections. The possibility that we were dealing with syphilitic lesions in the aorta and rheumatic lesions in the other locations was considered but dismissed because the lesions in all locations were histologically identical.

The possibility that we were dealing with lesions peculiar to and specific for rheumatoid arthritis was considered because of the striking similarity of the lesions described to those of the subcutaneous nodules of rheumatoid arthritis (fig. 9). Support for this point of view was obtained from a recent statement by Bennett, Zeller and Bauer [4] that the subcutaneous

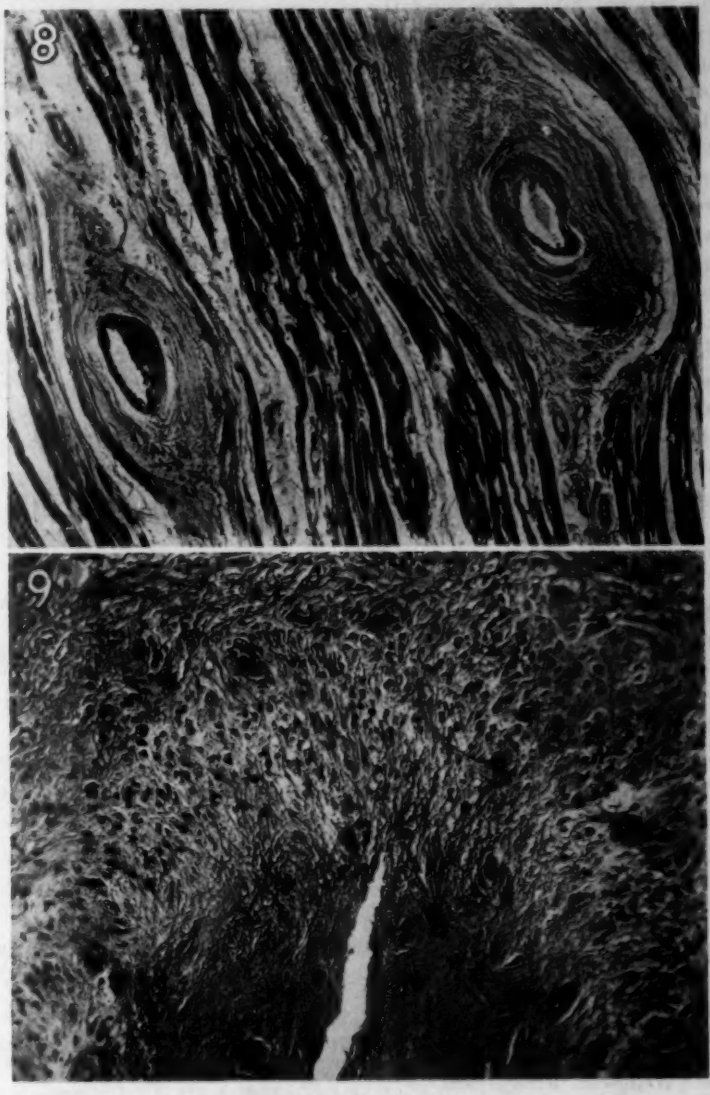


Fig. 8 (case 2).—Perivascular scars in the myocardium (hematoxylin and eosin; × 80).

Fig. 9.—Subcutaneous nodule from another patient's elbow for comparison with the cardiac lesions of the previous patients (hematoxylin and eosin; × 165).

4. Bennett, G. A.; Zeller, J. W., and Bauer, W.: Arch. Path. **30**:70, 1940.

nodules of rheumatoid arthritis and rheumatic fever differ as much from one another as do the granulomas of syphilis and tuberculosis. A careful comparison of the cardiac lesions in these 2 cases with typical rheumatic cardiac lesions discloses that the only significant distinguishing feature of the lesions in these 2 cases was the larger amount of central necrosis. The palisading of large cells of the intermediate zone and the peripheral nonspecific inflammatory reaction are also seen in typical rheumatic lesions, although not to such a pronounced degree. Necrosis also occurs in cardiac lesions of rheumatic fever (Swift [5]; Clawson [6]; Klinge [7]; Jaffé [8]). In the usual cardiac lesions of rheumatic fever, however, necrosis is not a prominent feature. The unusual amount of necrosis and the associated connective tissue reaction in these 2 cases may be a manifestation of an unusually large or potent etiologic agent, or they may indicate unusual sensitization or hyperergy of the tissues. The chronicity of the lesion and the pronounced arteriosclerosis with obliteration of the lumens of the vessels may also have played a role. According to Klinge,[7] the "fibrinoid degeneration" of the collagen in rheumatic lesions usually does not lead to complete necrosis, since the collagen fibrils can be demonstrated by silver impregnation. He admitted, however, that actual necrosis does occur, especially in the larger lesions of rheumatic fever. In our cases, also, intact collagen fibrils often could be demonstrated in the apparently necrotic central portions of the lesions although this was not always true.

It would seem, therefore, that in regard to the histologic differences between these lesions and the lesions of typical rheumatic fever we are dealing with differences of degree rather than of kind. Substantially the same point of view had been held with regard to the subcutaneous nodules of rheumatic fever and rheumatoid arthritis by Coates,[9] Clawson and Wetherby,[9a] Dawson,[10] Klinge [8] and Hawthorne.[11] Bennett, Zeller and Bauer [4] also stated that, although it is possible in most instances to distinguish the nodule of rheumatoid arthritis from that of rheumatic fever, it is readily apparent that the two present similar or identical structural and cytologic features. Also in favor of the rheumatic nature of these lesions were the facts that structures similar to Aschoff bodies occurred in the pericardium and the myocardium in case 1 and that in case 2 perivascular scars, which have been interpreted as healed Aschoff bodies, were present.

Because of all these considerations, we believe that the lesions described can be brought under the classification of rheumatic heart disease and doubt that they represent a separate disease entity.

Since most of the cardiac lesions associated with rheumatoid arthritis are indistinguishable from those produced by rheumatic fever (Baggenstoss and Rosenberg [1]; Andrus [12]; Fingerman [13]; Bayles [14]; Dawson [15]; Smyth [16]), these 2 cases are significant in demonstrating that the heart in some instances of rheumatoid arthritis may respond in a manner almost identical with that of the subcutaneous tissues.

SUMMARY

In 2 cases of multiple rheumatoid arthritis unusual cardiac lesions were noted which were strikingly similar histologically to the subcutaneous nodules of rheumatoid arthritis. Although most of the cardiac lesions associated with rheumatoid arthritis are indistinguishable from those produced by rheumatic fever, the cardiac lesions in these 2 cases indicate that in this disease the heart may occasionally react in the same manner as the subcutaneous tissues. Since the differences between the cardiac lesions described and those of typical rheumatic fever are differences of degree rather than of type, we believe that the lesions can be considered a manifestation of rheumatic heart disease.

5. Swift, H. F.: Am. J. M. Sc. **170**:631, 1925.
6. Clawson, B. J.: Arch. Path. **8**:664, 1929.
7. Klinge, F.: Klin. Wchnschr. **9**:586, 1930
8. Jaffé, R. H.: The Reticulo-Endothelial System, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 2, p. 1059.
9. Coates, V.: M. J. & Rec. **133**:55, 1931.
9a. Clawson, B. J., and Wetherby, M.: Am. J. Path. **8**:283, 1932.
10. Dawson, M. H.: J. Exper. Med. **57**:845, 1933.
11. Hawthorne, C. O.: Brit. J. Rheumat. **1**:109, 1938.
12. Andrus, F. C.: Minnesota Med. **24**:1071, 1941.
13. Fingerman, D., in discussion on Andrus.[12]
14. Bayles, T. B.: Am. J. M. Sc. **205**:42, 1943.
15. Dawson, M. H., in discussion on Rosenberg, E. F.; Baggenstoss, A. H., and Hench, D. S.: Ann. Int. Med. **19**:114, 1943.
16. Smyth, C. J., in discussion on Rosenberg, E. F.; Baggenstoss, A. H., and Hench, P. S.: Ann. Int. Med. **19**:114, 1943.

GENESIS OF THE MULTINUCLEATED GIANT CELLS IN THE LYMPHATIC TISSUE OF THE APPENDIX IN MEASLES

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The origin of measles giant cells from lymphatic tissue has been mentioned by several authors, but an inclusive discussion of the evolution of these cells has not been found. Another name for the type cell is the Warthin-Finkeldey giant cell, after the two investigators who described it independently in 1931 as observed in tonsils removed during measles. The purposes of this paper are: (1) to summarize and analyze the reported cases of measles in which a clinical history was available, in which clinical evidence of measles was definite and in which the appendix was subjected to pathologic examination and revealed giant cells corresponding to those described by Warthin [1] and Finkeldey [2]; (2) to record an additional instance of multinucleated giant cells in the appendix of a boy who underwent appendectomy with a mistaken clinical diagnosis of acute appendicitis in the prodromal stages of measles; (3) to formulate a reasonable hypothesis of the origin and evolution of the giant cells based on a histologic study of the appendix in this new case.

REVIEW OF THE CASES IN THE LITERATURE

Herzberg [3] reported the case of a child 6 years old in whom a measles rash developed four days after removal of the appendix for subacute appendicitis. Many multinucleated giant cells were found in the hyperplastic lymph follicles.

Finkeldey [4] described the case of a boy 8 years old on whom a measles rash was seen two days after appendectomy for acute diffuse appendicitis. Multinucleated giant cells were numerous in the germinal centers of the appendical lymphatic tissue.

Davidsohn and Mora [5] discussed 3 cases. The patients were boys 6, 8½ and 6 years old. In the first and third the rash of measles appeared two days after and in the second two days before appendectomy. Histologic examination in the first case disclosed acute suppurative appendicitis and numerous multinucleated giant cells in the exhausted lymph follicles and subepithelial layer; in the second, acute diffuse suppurative appendicitis and small numbers of these giant cells in the same locations, and in the third, acute diffuse suppurative appendicitis and numerous giant cells with three to four to thirty or more nuclei in the lymphatic tissue. In a fourth case, that of a boy 7 years old, no giant cells were discovered, but acute diffuse suppurative appendicitis and periappendicitis were seen nine days after the outbreak of a measles rash.

The appendix from the 6 year old boy reported by Fischer [6] showed enterobiasis and numerous giant cells with six to eight to twenty or more nuclei in the

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1. Warthin, A. S.: Arch. Path. **11**:864, 1931.
2. Finkeldey, W.: Virchows Arch. f. path. Anat. **281**:323, 1931.
3. Herzberg, M.: J. A. M. A. **98**:139, 1932.
4. Finkeldey, W.: Virchows Arch. f. path. Anat. **284**:518, 1932.
5. Davidsohn, I., and Mora, J. M.: Arch. Path. **14**:757, 1932.
6. Fischer, W.: Beitr. z. path. Anat. u. z. allg. Path. **91**:474, 1933.

germinal centers of the lymph follicles. One and one-half days after operation a measles rash was evident.

Schultze [7] found fibrosis of the serosa and many giant cells with ten or more nuclei at the edges of the lymph follicles and in the subepithelial layer of the appendix of a 10 year old girl who broke out with a measles rash two days after appendectomy.

Wegelin [8] delineated the histologic changes in the appendixes of 3 children. The amount of inflammatory reaction in each warranted the diagnosis of mild acute appendicitis. In the germinal centers, in the surrounding lymphatic tissue and in the subepithelial layer were large numbers of Warthin-Finkeldey giant cells, some with fifty to one hundred nuclei. The first patient was a boy 8 years old; the second and third, girls 8 and 6½ years old. The boy had Koplik spots three days and measles rash four days after operation; of the girls, the first had Koplik spots two days and measles rash three days after operation, and the second, a measles rash on the day of operation.

The 5 year old boy described by Newman and Milstead [9] acquired Koplik spots and a measles rash three days after operation. The appendix disclosed fibrosis of the submucosa and serosa and many multinucleated giant cells in the lymph follicles and mucosa.

Hathaway [10] reported the case of a boy 2½ years old who died four days after exposure to a patient with measles, without showing Koplik spots or a measles rash. Autopsy revealed acute suppurative appendicitis with perforation and general fibrinopurulent peritonitis. No multinucleated giant cells were seen in the appendix, but many were present in the lymph nodes and spleen.

A child 4 years old, described by Gräff,[11] died fifteen days after exposure to a patient with measles, without showing Koplik spots or a measles rash. Necropsy disclosed many multinucleated giant cells in the tonsils and cervical lymph nodes but none in the appendix or other organs.

In a review of appendicitis in the exanthems Bullowa, McCabe and Wishik [12] included a histologic description of three appendixes removed from children during the preeruptive period of measles because of symptoms of pseudoappendicitis. These appendixes showed characteristic giant cells in the mucosal and submucosal layers. A definite statement about the presence of concomitant acute appendicitis was not made, nor were details of the clinical histories of these three children given.

Masugi and Minami [13] described the case of a girl 9 months old who died from bronchopneumonia four days after the appearance of the rash of measles. In the mucous membranes of the mouth, pharynx, esophagus, trachea, bronchi and bronchioles were many multinucleated giant cells with acidophilic inclusion bodies. These authors thought that the giant cells were derived from the epithelial cells of the mucous membranes. No giant cells were seen in the lymphatic tissue of the alimentary canal, including the appendix, or in the spleen, the lymph nodes or the thymus. I agree with Stryker [14] that the giant cells described by Masugi

7. Schultze, W. H.: München. med. Wchnschr. **80**:576, 1933.
8. Wegelin, C.: Schweiz. med. Wchnschr. **67**:1, 1937.
9. Newman, P. F., and Milstead, L. C.: J. Internat. Coll. Surgeons **3**:551, 1940.
10. Hathaway, B. M.: Arch. Path. **19**:819, 1935.
11. Gräff, S.: Deutsche med. Wchnschr. **63**:1357, 1937.
12. Bullowa, J. G. M.; McCabe, E. J., and Wishik, S. M.: Am. J. Dis. Child. **53**:1029, 1937.
13. Masugi, M., and Minami, G.: Beitr. z. path. Anat. u. z. allg. Path. **101**:482, 1938.
14. Stryker, W. A.: Am. J. Dis. Child. **59**:468, 1940.

and Minami were not the same as those originally depicited by Warthin [1] and Finkeldey [2] as characteristic of measles.

Semsroth [15] discussed the case of a boy 20 months old who was twice exposed to measles in the course of two weeks. Fourteen days after the first exposure and two hours after an unsuccessful attempt at bronchoscopic examination, severe dyspnea developed and the child died without showing Koplik spots or a measles rash. The palatine tonsils, the mediastinal lymph nodes, the spleen, the thymus and the epithelium of the tongue, tonsillar crypts and bronchi contained multinucleated giant cells with cytoplasmic inclusion bodies. Pathologic examination of the gastrointestinal tract, including the appendix, was not mentioned.

Stryker [14] reported the case of a girl 27 months old who died after a probable exposure to measles without showing Koplik spots or a measles rash. At autopsy multinucleated giant cells characteristic of measles were seen in the lungs, the spleen and the lymph nodes. The appendix was not examined.

REPORT OF A CASE

A boy 5 years old was admitted to the Colorado General Hospital Feb. 26, 1943. For a year and a half he had had attacks of abdominal pain and vomiting at intervals of two

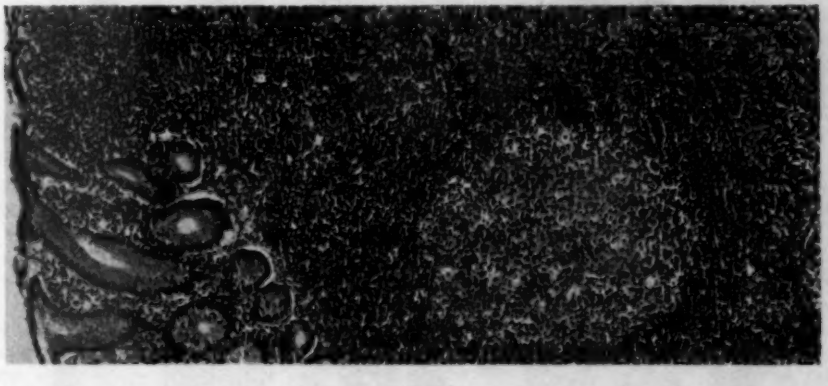

Fig. 1.—Section of appendix. The lumen is at the extreme left. Columnar epithelium covers the surface of the mucosa and lines the glandular crypts in the left one third. Lymphatic tissue with an active secondary lymphatic nodule makes up the right two thirds. Note four giant cells in the upper right corner of the active secondary lymphatic nodule. × 70.

or three months. Sixteen months before, his tonsils and adenoids had been removed. For one week he had a cold. For three days he had epigastric pain which localized in the right lower quadrant of the abdomen. One day before admission he vomited four times; twelve hours before, he had inferior subcostal pain on the right side and expectorated bloody sputum; three hours before, his temperature was 103 F.

On admission the temperature was 100 F.; the pulse rate, 100. The nose and throat were congested and dripping. A systolic murmur was heard over the mitral area of the heart. The abdomen revealed spasm of the right rectus muscle and rebound tenderness in the right lower quadrant. Digital examination indicated tenderness on the right side of the rectum. The admission diagnosis was acute appendicitis, infection of the upper respiratory tract and pneumonia.

The hemoglobin content (Newcomer) was 13.5 Gm.; the erythrocyte count, 5,000,000; the total leukocyte count, 5,000, with polymorphonuclear neutrophils 67 per cent, lymphocytes 24 per cent, monocytes 8 per cent and basophils 1 per cent. The urine contained albumin (a trace) and acetone (3 plus). A roentgenogram of the chest showed no abnormality. Cultures of material from the nose and the throat revealed diphtheroids.

15. Semsroth, K. H.: Arch. Path. **28**:386, 1939.

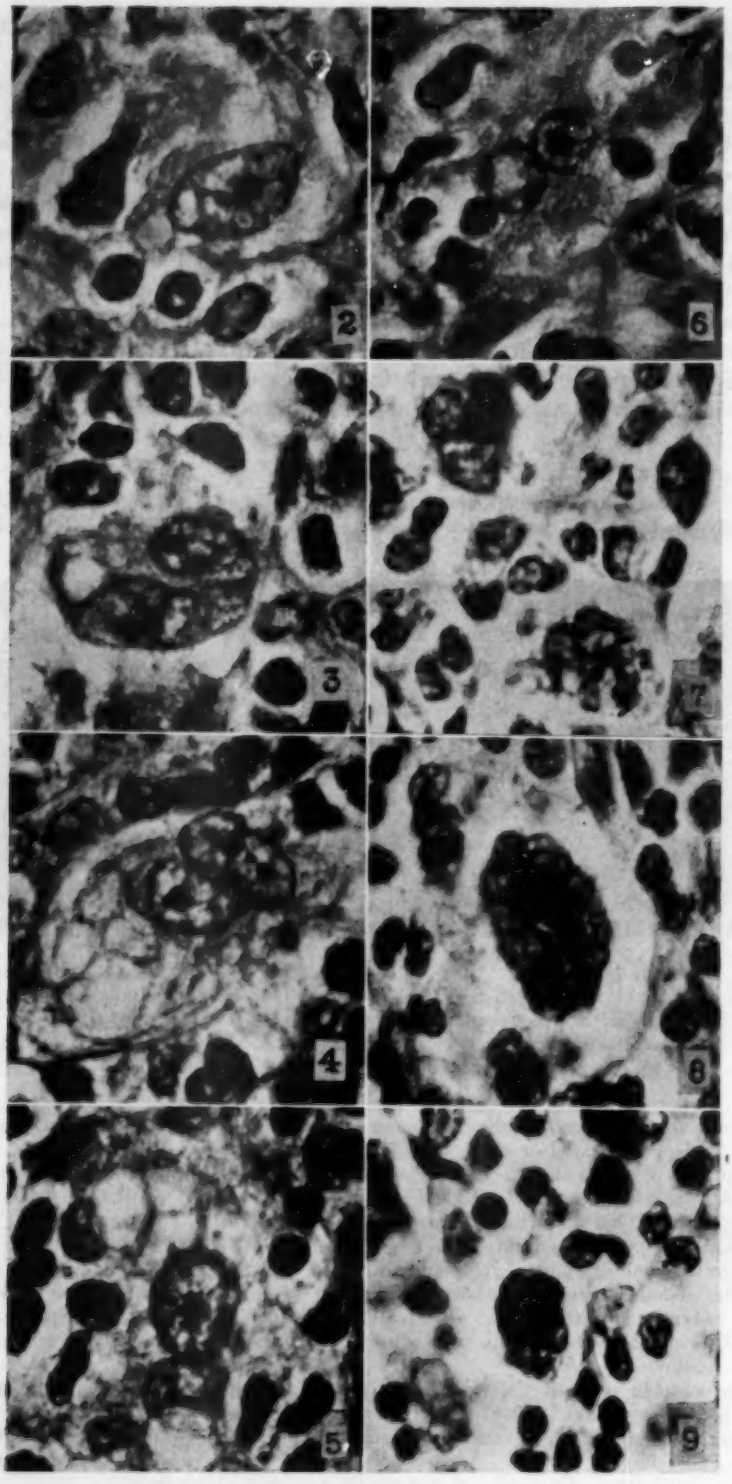

Figure 2
(See legend on opposite page)

Within three hours after admission, with the patient under anesthesia induced with cyclopropane, the appendix was removed through an incision splitting the rectus muscle. No gross abnormalities of the peritoneum, the abdominal viscera or the appendix were observed.

One day after operation the temperature spiked to 105 F. Koplik spots were found in the buccal lining. Two days later the temperature again spiked to 105 F. and a rash typical of measles appeared. The temperature dropped by lysis to normal on the sixth day and remained normal. The sutures were removed on the eighth day. On the twelfth day the rash was faded, but blepharitis was present. This was treated with boric acid compresses and an ointment containing yellow mercuric oxide. The blepharitis had largely subsided and the appendectomy wound was well healed when the patient was discharged fifteen days after admission and operation.

The appendix was 8 cm. long and 5 mm. in diameter, with a pink-gray smooth serosa, a thin white wall, a yellow intact mucosa and a patent lumen. Microscopic examination was made on longitudinal sections through the distal third and transverse sections through the middle and proximal thirds after fixation in 4 per cent solution of formaldehyde, embedding in paraffin, cutting at 6 microns and staining with hematoxylin and eosin. Proper material was not available for Giemsa and scarlet red stains.

The lumen of the proximal two thirds was empty. In the lumen of the distal third were two well developed female worms, Enterobius vermicularis. The columnar epithelium covering the mucosa and lining the glandular crypts was intact. As many as four mitotic figures were present in the epithelium of the glandular crypts. The lymphatic tissue (fig. 1) was abundant and revealed about one active secondary lymphatic follicle per low power field (× 125). In these secondary follicles several cell types were seen, including the following: (1) stem cells with a large oval nucleus enclosing a fine chromatin net, one or more acidophilic nucleoli and pale acidophilic cytoplasm with irregular blunt processes; (2) lymphoblasts with a medium-sized nucleus enclosing a fine focally condensed chromatin net, an acidophilic nucleolus and a rim of acidophilic cytoplasm; (3) lymphocytes with a small round nucleus enclosing condensed non-netted chromatin, and scanty pale acidophilic cytoplasm; (4) prolymphocytes intermediate in nuclear and cytoplasmic development between lymphoblasts and lymphocytes. Also present were a few mitotic figures and scattered, free, deeply basophilic fragments, probably broken-down nuclei.

In addition to the cell types described, in the active secondary follicles was seen a series of giant cells which resembled in their development the lymphocyte series. The youngest forms (fig. 2) contained one large oval nucleus with a fine chromatin net, one or more nucleoli and abundant irregular acidophilic vacuolated cytoplasm with blunt processes. The next stages in maturation included the bilobed segmentation of the nucleus (fig. 3), division by amitosis into two nuclei (fig. 4) and nuclear condensation and rounding (figs. 5 and 6). The cytoplasm retained the pale acidophilia and the irregular blunt processes but became smaller in volume and less vacuolated. Amitotic division progressed through the stages of four and eight nuclei (fig. 7). The nuclear chromatin was more condensed, the nucleoli were less prominent or absent, the focusing of nuclei for photomicrography was difficult, and the cytoplasm became more scanty. The next division by amitosis was into at least sixteen nuclei and perhaps more (fig. 8). The nuclear chromatin was almost completely condensed, individual nuclei were difficult to outline completely, and the cytoplasm was scanty. The final stage of giant cell formation was seen only in the tissue around the active secondary follicles. These cells (fig. 9) contained at least eight nuclei with completely condensed

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EXPLANATION OF FIGURES 2 TO 9

Fig. 2.—Stem cell. × 1,000.

Fig. 3.—Bilobed stem cell in an early stage of amitotic division. × 1,000.

Fig. 4.—Binucleated young giant cell. × 1,000.

Figs. 5 and 6.—Binucleated giant cells showing nuclear rounding and condensation of nuclear chromatin. × 1,000.

Fig. 7.—Giant cell with four nuclei in the upper left corner (one nucleus is out of focus). Giant cell with eight nuclei in lower right corner (four nuclei are easily visible, two nuclei are out of focus and two nuclei are not visible). × 1,000.

Fig. 8.—High power magnification of the largest giant cell visible in figure 1. Note the multiple condensed nuclei, some visible, some out of focus. × 1,000.

Fig. 9.—Giant cell present in lymphatic tissue around active secondary lymphatic nodules. Note the nuclear condensation. Lymphocytes are nearby. × 1,000.

chromatin and had scanty cytoplasm. They represented partly degenerated forms of the giant cells or the end stage of their evolution through splitting of the forms with sixteen or more nuclei. The submucosa, the muscle coats and the serosa were intact.

COMMENT

The presence of giant cells in the appendix in the cases of measles described by Herzberg,[3] Finkeldey [4] and Davidsohn and Mora [5] was complicated by varying degrees of inflammatory reaction. In the other cases [16] in which multinucleated giant cells were found, appendectomy was not justified if the paucity of histologic inflammatory changes is a criterion. This criticism also applies to the reported case, which is a clearcut example of pseudoappendicitis in measles. The acute nasopharyngitis, the cough, the bloody sputum, the temperature of 103 F. before admission and the total leukocyte count of 5,000 favored the diagnosis of prodromal measles. However, the attacks of vomiting, the epigastric pain localizing in the right lower quadrant of the abdomen, the admission temperature of 100 and the spasm and tenderness of the right side of the abdomen argued for acute appendicitis, which may be seen concomitantly with measles.[17] In addition, since the total leukocyte count is not always an accurate index of acute appendicitis, since an acute infection of the upper respiratory tract may accompany and may mask acute appendicitis and since the diagnosis of appendicitis in children is more difficult than that of appendicitis in adolescents or young adults, I hold that appendectomy in the reported cases and in the new case was entirely justified. This is particularly true in view of the relatively small number of recorded instances of appendectomy for actual or simulated appendicitis in a patient with measles. More histologic studies on the appendixes of children with pseudoappendicitis in measles should be found in the literature if this diagnostic mistake were frequent. No sure method for avoiding it is offered. I agree with those surgeons who believe that it is better to operate on 10 children if acute appendicitis is caught in an early stage in only 1, the other 9 showing little or no inflammatory changes in the appendix, provided acute appendicitis figures prominently in the preoperative differential diagnosis. This opinion is further strengthened by the all too conspicuous place which appendicitis still occupies as a cause of death in the general population.

The development of the multinucleated giant cells in the appendix in the reported cases is suggested in part by several authors. In describing the histologic changes in the tonsils removed from 4 children with measles, Warthin [1] stated, "One received a definite impression that the giant cells arose by amitotic division in hyperchromatic cells resembling lymphocytes in the subepithelial reticulo-endothelial layer and in the germinal centers, and wandered toward and into the mucosal epithelium." Finkeldey [2] considered the possibility that the giant cells might represent a fusion of lymphocytes or might be derived from a kind of undifferentiated mesenchymal cell as well as from reticulum cells. Herzberg [3] expressed the opinion that the multinucleated giant cells might be formed of aggregations or coalescent cells of lymphoid origin. In regard to the histologic description of the giant cells in the appendix in the third case of Davidsohn and Mora,[5] Dr. Otto Saphir said, "The nuclear chromatin was abundant and coarsely trabeculated, strongly suggesting lymphocytic or plasma cell nuclei." In the appendix of his patient Fischer [6] described nuclei in the giant cells like the nuclei of small lymphocytes. He also stated that some of the nuclei were larger and clearer, corresponding to the nuclei of reticulum cells. With sudan stain he was able to demonstrate fine droplets of

16. Fischer.[6] Schultze.[7] Wegelin.[8] Newman and Milstead.[9]
17. Herzberg.[3] Finkeldey.[4] Davidsohn and Mora.[5]

neutral fat in the cytoplasm of the giant cells. Wegelin [8] expressed the opinion that the giant cells were derived for the most part from the reticulum but that those in the lamina propria of the mucous membrane could have originated from lymphoblasts and lymphocytes. Hathaway [10] said, "They appeared to have arisen from the reticulo-endothelial cells of the sinuses," in commenting on the origin of the giant cells in the lymph nodes and the spleen in his case. Semsroth [15] suggested that the multinucleated giant cells were formed through amitotic division of the nuclei of plasma cells. It is suggested that the inoculation of cultures of lymphatic tissue with material from patients with active measles might serve to elucidate the genesis of the multinucleated giant cells. In the prodromal stage of measles the differentiation of acute appendicitis from pseudoappendicitis may be difficult or impossible even after careful consideration of the history and of the physical and laboratory findings.

SUMMARY

The authors who described the cases of measles in which multinucleated (Warthin-Finkeldey) giant cells were found in the lymphatic tissue of the appendix considered the possibility that these originated from amitotic division of cells resembling lymphocytes, from fusion of lymphocytes, from undifferentiated mesenchymal cells, from reticulum cells and from amitotic division of plasma cells. On the basis of the histologic description of the appendix in the new case, the suggestion is offered that the multinucleated giant cell of the lymphatic tissue in measles is formed as a result of the polynuclear abnormal development of the stem cell paralleling the mononuclear normal development of the lymphocyte from the stem cell.

Laboratory Methods and Technical Notes

HEMATOXYLIN-EOSIN TISSUE STAIN
An Improved, Rapid and Uniform Technic

EDWIN E. ZIEGLER, M.D., BETHLEHEM, PA.

The improved staining technic presented herewith will give identical results in the hands of any technician.

It is generally conceded that with present methods there is considerable variation in the results obtained with the hematoxylin-eosin tissue stain. In spite of this variation the stain remains a popular one.

The chief cause of the variable and sometimes unsatisfactory results is the indefinite method of decolorizing or differentiating the sections after the staining in hematoxylin.

After the staining in hematoxylin the present art [1] calls for washing in several changes of tap water over a period of several hours or overnight, or in running tap water for ten to thirty minutes or, if overstaining has occurred, for decolorizing in 1 per cent alum water, 1 per cent acetic acid or acid alcohol, followed by thorough washing in tap water. There are those who use routinely a 1 or 2 per cent aqueous solution of hydrochloric acid, followed by ammonium hydroxide.

The color and the degree of differentiation obtained by washing in tap water depends on the length of time the section is washed, on the p_H of the water and probably on other characteristics of the water, including its chemical composition. If a section is left in an acid solution, it will become pale or the blue color of the hematoxylin may be bleached out.

If the section is treated in an acid bath, it must then be alkalinized, usually by prolonged washing in slightly alkaline tap water or in an alkaline solution such as an ammonium hydroxide or a lithium carbonate solution.

The procedures of acid treatment, alkaline treatment and prolonged washing are the ones which require experience and make the process an art, the results of which are more or less variable from time to time even in the hands of the same technician.

The following technic is the one that my coworkers and I are now using after two years of experimentation.

From the Pathological Laboratory, St. Luke's Hospital.

1. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938, pp. 71 and 72. Cowdry, E. V.: Microscopic Technique in Biology and Medicine, Baltimore, Williams & Wilkins Company, 1943, p. 93. McClung, C. E.: Handbook of Microscopical Technique, ed. 2, New York, Paul B. Hoeber, Inc., 1937.

TECHNIC

After cutting sections and fixing them on slides proceed as follows:

1. Dip eight times in xylene.
2. Dip eight times in second xylene.
3. Dip eight times in absolute alcohol.
4. Dip eight times in second absolute alcohol.
5. Dip eight times in 95 per cent alcohol.
6. Dip eight times in isotonic solution of sodium chloride.
7. Stain in properly ripened alum-hematoxylin for six minutes (Delafield's or Harris').
8. Dip eight times in isotonic solution of sodium chloride.
9. Dip four times in 2 per cent solution of phosphotungstic acid in isotonic solution of sodium chloride (about four seconds).
10. Dip eight times in isotonic solution of sodium chloride.
11. Dip four times in 2 per cent solution of sodium citrate in isotonic solution of sodium chloride (about four seconds).
12. Dip eight times in isotonic solution of sodium chloride.
13. Counterstain two minutes in 5 per cent eosin in a 50 per cent mixture of ethyl alcohol and distilled water.
14. Dip eight times in 95 per cent alcohol.
15. Dip eight times in absolute alcohol.
16. Dip eight times in second absolute alcohol.
17. Dip eight times in xylene.
18. Dip eight times in second xylene.
19. Mount in balsam.

It is advisable to use eighteen glass staining jars with covers to prevent evaporation of solutions. We use standard size jars that accommodate a metal rack having a wire handle, which holds up to thirty slides. As solutions evaporate, they may be added to. The saline washes should be changed more frequently than the other solutions. A common wash bath in place of those in the series is not advisable.

This technic is continuous and rapid and gives uniform results. Timing should be as stated and not according to gross color as in other technics. The entire procedure can be done in less than fifteen minutes, not counting mounting.

With this technic the following results are obtained: nuclei, blue; cytoplasm, pink to lilac; muscle tissue, lavender; fibrous tissue, pink; elastic lamina of blood vessels, brilliant pink; nerve tissue, light grayish purple. In general, fibers of all kinds are well stained. Necrotic substances stain various shades of purple or pink. The technic gives a good contrast between blue-purple shades and the pink counterstain shades. At the same time there is a greater range of

tinting of the various elements of the tissue. Sections stained with this technic have now been in the files for over a year. They show no fading. The method and the result do not resemble those of Mallory's phosphotungstic acid–hematoxylin stain.

Tissues fixed in Zenker's fluid may require more time in the hematoxylin. We have obtained best results with tissues fixed in a 10 per cent dilution of a commercial preparation of solution of formaldehyde U. S. P. We have recently been using a 5 per cent dilution of this preparation for small blocks or specimens, with good results (an economy measure). Fixatives containing an acid, such as acetic acid, do not give as good results.

Timing in hematoxylin may vary somewhat, depending on the dye and its preparation, as well as on its degree of ripeness. A properly prepared and ripened stain will give good results in four to eight minutes. This must be determined for the particular stain in use.

Hematoxylin solutions to which acid of some kind is added do not give good results. If an acid fixative is used, there should be thorough washing before staining.

The solution of phosphotungstic acid used in the staining process decolorizes or differentiates the hematoxylin stain in the tissue to the right degree and then stops. Three or four dips of the slides in the solution are sufficient. More time devoted to this is superfluous. If more time is used, no harm is done. We have timed tissues in this solution up to five minutes and only the slightest additional bleaching can be noticed.

The use of phosphotungstic acid for this purpose was discovered in the course of trying twenty-seven different acids or acid salts. The p_H of these substances is important but is not the whole story. It ranged from p_H 6.9 down to a p_H value of less than 1. All of them produce differentiation. Many of them, like the strong acids, are hard to control, act too rapidly and must be timed according to gross color. Several were found which would differentiate the hematoxylin to a certain degree and then stop. Of these, phosphotungstic acid gave the best results. Following is the list of chemicals tried. All were used empirically in a 2 per cent solution or in a saturated solution if solubility was less than this. A complete set of autopsy sections was treated with each chemical in turn.

Acids	Acid Salts
Lactic	Mercury bichloride
Citric	Magnesium sulfate
Phosphotungstic	Cupric sulfate
Acetic	Ferric chloride
Phosphoric	Cuprous chloride
Picric	Ammonium molybdate
Tartaric	Ammonium nitrate
Paratoluene sulfonic	Ammonium sulfate
Sulfanilic	Ammonium bromide
Formic	Potassium persulfate
Tannic	Phenylsalicylate
Uric	
Sulfuric	
Hydrochloric	
Nitric	
Molybdic	

The use of sodium citrate in the series is also new. It takes the place of prolonged washing in tap water. It obviates the use of stronger alkaline substances, such as lithium carbonate or ammonium hydroxide. We experimented with solutions of various alkaline salts, including sodium bicarbonate and disodium phosphate. Sodium citrate gave the best results.

The 2 per cent sodium citrate solution neutralizes any excess of phosphotungstic acid in the tissue section. It conditions the tissue in some way and gives it the proper p_H. This results in the desired degree of counterstaining with eosin. Slides were counterstained in the eosin solution for intervals varying from thirty seconds to five minutes. They all showed about the same degree of staining. The timing in the eosin solution is therefore uncritical.

SUMMARY

A new technic for the routine hematoxylin-eosin tissue stain is presented which is rapid, requiring less than fifteen minutes. There is little possibility of overstaining or understaining with either hematoxylin or eosin. The technic provides good color contrasts as well as excellent differentiation. The colors are usually more brilliant than those obtained with other methods.

Case Reports

PRIMARY SARCOMA OF THE HEART

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Primary tumor of the heart is still uncommon enough to justify the description of any new case. According to Mandelstamm,[1] 143 cases had been published up to 1923. In 1931 Yater [2] brought the total to 150, and in 1941 Lisa, Hirschhorn and Hart [3] added 33 more cases.

Certain conclusions have been drawn from these observations: the more frequent occurrence of noncancerous than of cancerous growths; the greater incidence of the former in the left auricle and ventricle in contrast to the latter, which are mostly encountered in the right auricle; the predominance of rhabdomyoma among the noncancerous growths and of sarcoma among the cancerous, respectively, over the other less frequent histologic types—these are established facts.

In contrast to sarcoma, which is mostly seen in the aged, the finding of rhabdomyoma in the newborn, in association with other malformations, led to the conception of rhabdomyoma as congenital.

Among the concomitant findings, tuberous sclerosis of the brain, glioblastomatosis, cleft palate, harelip, cystic liver or kidney and sebaceous gland adenoma have been mentioned most frequently. Yet, in describing the fifty-first case of congenital rhabdomyoma of the heart, associated with cardiac developmental defects, spongioblastoma and tuberous sclerosis of the brain, Labate [4] pointed out the divergency still present between the supporters of the true neoplastic nature of the growth (Wolbach [5]) and those in favor of its essentially malformative character (Steinbiss [6]; Redher [7]; Schmincke [8]). For a critical review of this controversial point, the reader is referred to Farber's article.[9]

The other outstanding group of cardiac growths is more directly related to the histologic features of the case I have recently been privileged to study. According to Perlstein [10] 31 cases of primary sarcoma of the heart had been published up to 1918. Fifteen more cases were listed independently by Diebold [11] in 1930 and by Yater [2] in 1931, whereas Weir and Jones [12] made the total of cases 75 to January 1940. Of the 75 neoplasms, 19 were diagnosed as round cell sarcoma, 17 as spindle cell sarcoma, 7 as fibrosarcoma, 6 as myxosarcoma, 5 as angiosarcoma, 5 as mixed cell sarcoma, 4 as giant cell sarcoma, 2 as lymphosarcoma and 1 each as rhabdomyosarcoma and leiomyoblastic sarcoma; eight were not classified. In the case described by Weir and Jones the growth involved the right atrium. Outstanding microscopic features were highly undifferentiated cells resembling both epithelial and reticulum cells, interposed with spindle-shaped elements showing sausage-shaped nuclei and at times fibrillated cytoplasmic processes; therefore the diagnosis of leiomyosarcoma was made.

From the laboratories of the Medfield State Hospital.

1. Mandelstamm, M.: Virchows Arch. f. path. Anat. **245**:43, 1923.
2. Yater, W. M.: Arch. Int. Med. **48**:626, 1931.
3. Lisa, J. R.; Hirschhorn, L., and Hart, C. A.: Arch. Int. Med. **67**:91, 1941.
4. Labate, J. S.: Am. J. Path. **15**:137, 1939.
5. Wolbach, S. B.: J. M. Research **16**:495, 1907.
6 Steinbiss, W.: Virchows Arch. f. path. Anat. **243**:22, 1923.
7. Redher, H.: Virchows Arch. f. path. Anat. **217**:174, 1914.
8. Schmincke, A.: Beitr. z. path. Anat. u. z. allg. Path. **70**:513, 1922.
9. Farber, S.: Am. J. Path. **7**:105, 1931.
10. Perlstein, I.: Am. J. M. Sc. **156**:214, 1918.
11 Diebold, O.: Ztschr. f. Kreislaufforsch. **22**:785, 1930.
12. Weir, D. R., and Jones, B. C.: Am. Heart J. **22**:556, 1941.

Subsequent to these observations are those of Haythorn, Ray and Regis [13] and the 2 observations of Weller.[14] In the case of Haythorn, Ray and Regis the tumor, which appeared microscopically as fibromyxosarcoma, was implanted at the root of the pulmonary artery and had given extensive pulmonary metastases. In both cases of Weller the tumor originated from the right auricle, and in both the histologic patterns suggested hemorrhagic sarcoma. There were large areas of necrosis and hemorrhage among masses of spindle-shaped cells, at times arranged in perivascular fashion. Compare with these cases the 2 previous observations of Choisser and Ramsay [15] in which newly formed blood vessels in great number, together with a growth of a variety of young mesenchymal cells embedded in a well organized reticulum, were thought to have originated from elements of the reticuloendothelial system. Both growths were diagnosed by the authors as angioreticuloendothelioma; Weir and Jones [12] in reviewing these 2 cases considered them as angiosarcoma.

The criteria on which the diagnosis of certain varieties of connective tissue tumor is based are not well established, mostly reflecting the uncertainties of classification of the connective tissue cells in general and of limiting and defining the reticuloendothelial system in particular.

Broad lines of division have been traced, some long ago and some more recently, between the various members of the sarcoma group. The wide acceptance and the daily application to practical histologic problems of the terms "lymphosarcoma," "Hodgkin's sarcoma," "Kaposi's sarcoma," "angiosarcoma," "reticulum cell sarcoma," "histiosarcoma," "round cell sarcoma" "spindle cell sarcoma," "polymorphous sarcoma" and "giant cell sarcoma" clearly attest the value of maintaining such subdivisions. Yet in view of the different diagnostic criteria by which the members of the group are classified by the authors, their existence as single entities is reasonably doubted. Occasional forms transitional from one type to another, recently emphasized by Gall and Mallory,[16] lend credence to the belief in an origin essentially common to all these tumors, the variations in patterns and intimate cytologic characteristics being explained on the basis of the degree of cellular differentiation.

To illustrate the difficulty in diagnosing such varying tumors, three well known pathologists who were consulted in the case I shall report expressed different opinions, equally well based, but leading to classification in different subdivisions of the sarcoma group.

REPORT OF A CASE

Fourteen years ago a 68 year old white man was admitted to the state hospital because of an abnormal mental condition. He was well developed and well nourished, talkative, euphoric, with frequent flights of ideas. There were varicosities of the legs and a small lipoma at the back of the neck. The blood pressure was 145 systolic and 90 diastolic. During his stay his condition remained the same, with some aggravation of the varicosities of the legs. In the last six months cyanosis developed which, although not particularly marked, remained definitely noticeable. The heart was enlarged to the right; the sounds, however, were clear, and the rate and the rhythm remained normal. The blood pressure at that time was 180 systolic and 100 diastolic. The clinical picture was one of rapid cardiac decompensation, and death occurred suddenly.

Autopsy.—The body was that of a well developed and rather well nourished white man about 68 years old. The face and neck had a peculiar dusky color, and the mucous membrane of the mouth and lips was deep pink. The veins of the head, neck and shoulders stood out prominently. There was marked telangiectasia of the face, and branching vessels were noticeable at the anterior aspect of the chest. Pitting edema was present around the ankles. A freely movable subcutaneous lipoma of the approximate size and shape of a tangerine was present at the posterior aspect of the neck.

13. Haythorn, S. R.; Ray, W. B., and Regis, A. W.: Am. J. Path. **17**:261, 1941.
14. Weller, G. L.: Ann. Int. Med. **14**:314, 1939.
15. Choisser, R. H., and Ramsay, E. M.: Am. J. Path. **15**:155, 1939.
16. Gall, E. A., and Mallory, T. B.: Am. J. Path. **18**:381, 1942.

The internal organs of the skull, the chest and the abdomen did not reveal any remarkable change except the heart. Two lymph nodes at the hilus of the left lung were each approximately the size of a cherry, soft in consistency and grayish white. The prostate was increased in size, with all the characteristics of simple hyperplasia.

The pericardial area filled most of the anterior mediastinum. No fluctuation was felt. The pericardium contained about 30 cc. of serosanguinous fluid. Cultures revealed inulin-fermenting enterococci and colon bacilli. The parietal pericardium was congested but free from adhesions except for a small area over the right auricle, in which were firm though easily broken adhesions. In this area, approximately 4 by 2 cm., the pericardium was somewhat thickened—to about 3 mm.—puckered, coarse and dotted with recent hemorrhages.

The heart (fig. 1) after removal of the blood content weighed 628 Gm. It measured 16 cm. in transverse diameter and 10.5 cm. in length. The epicardium was transparent, glistening and slightly congested. The usual shape of the heart was altered by an irregularly spherical mass arising from the right auricle. This mass measured 10 by 8.9 by 8.5 cm. Externally it

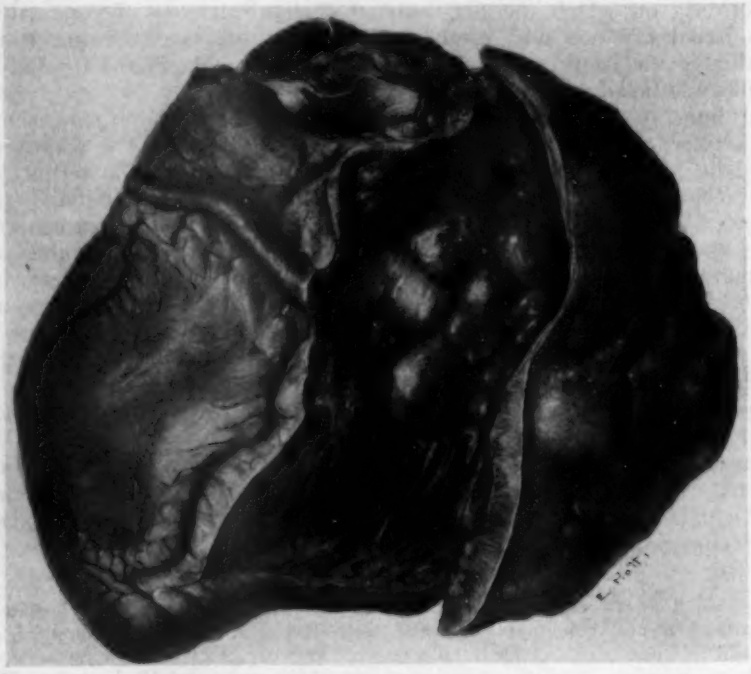

Fig. 1.—Polypoid tumor formations infiltrating the cavity of the right atrium.

was irregular owing to alternately bulged and depressed areas. The color ranged from gray to yellow, with interposed ecchymotic or larger hemorrhagic effusions. On the cut section it had a translucent appearance with a homogeneous grayish yellow tinge. Except for a large central area with evidence of advanced necrobiotic change, the consistency of the growth was firm.

There was another mass, similar to the one described but smaller, a few millimeters beneath the root of the aorta, measuring 3 by 2.5 by 1.2 cm. In the proximity of this growth a few enlarged periaortic lymph nodes were found, three on the anterior wall of the ascending portion of the aorta and three others deeply embedded in the loose connective tissue of the pulmonic-aortic intersection. All were about as large as peas, firm and grayish yellow.

The right auricle was greatly enlarged and almost completely filled with closely packed polypoid formations (fig. 1), which were firmly attached to the superior and posterior walls of the atrium and which hung down in the atrial cavity to the level of the insertion of the tricuspid leaflets. These polypoid formations were roughly pear shaped, soft, gray-pink, with smooth and glistening surfaces. They ranged in size from that of a pea to that of a cherry, a few reaching the size of a chestnut. Some were sessile and some pedunculated, the peduncles being at times independent of each other and at times united in a common broad base. The

leaflets of the tricuspid valve were completely surrounded by these formations, some of which projected in front of and some behind the leaflets; the leaflets themselves, however, appeared to be completely intact.

A polypoid mass approximately the size of a big cherry extended from the right auricle into the right auricular appendix, which was navicular in shape and greatly enlarged. It

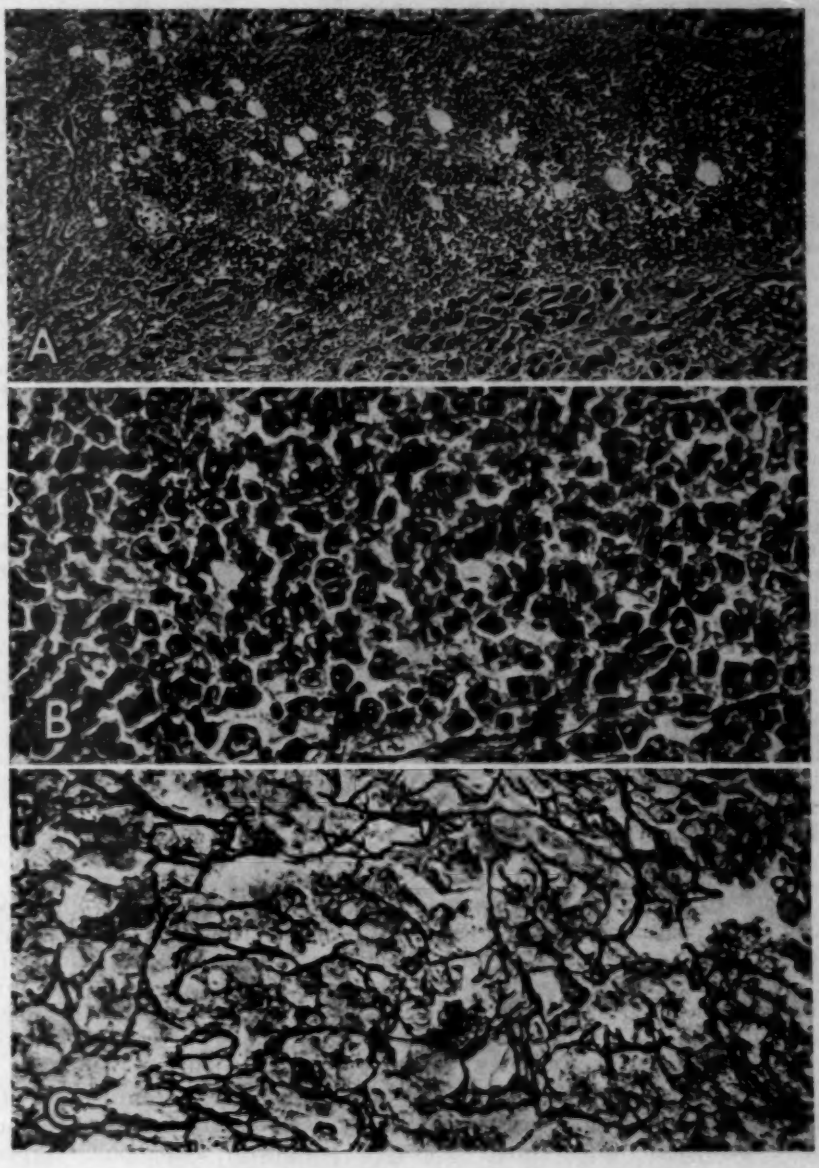

Fig. 2.—*A*, low power view of the neoplastic infiltration within the myocardium; × 100. *B*, tumor cell types seen in high magnification; × 475. *C*, network of argentaffin fibers in the bulk of the tumor, showing the delicate reticulum surrounding almost every cellular element; Foot stain; × 500.

measured 9 by 3.7 cm. and was surrounded by laminated clotted blood, leading to complete obliteration of the cavity.

The left atrial and ventricular cavities were slightly dilated. The aortic and mitral valves were intact. The pulmonic orifice, guarded by three thin, pliable, sharp-bordered cusps, was only 3.4 cm. in circumference, owing to compression of the opening by the main tumor mass. The aorta, just above its origin, was slightly enlarged and showed moderate intimal atheromatous change.

Microscopic Anatomy.—Tissue was fixed in a 4 per cent saline solution of formaldehyde and embedded in paraffin. The sections were stained with hematoxylin and eosin and with Giemsa stain. The silver impregnation method of Foot for precollagenous connective tissue was also used, and the sudan stain on frozen sections.

The outstanding feature of the main growth was a homogeneous distribution of cells (fig. 2 *A*) either singly or in closely approximated groups, with scarce intercellular material. Under higher magnification the cells were polygonal, oval or round (fig. 2 *B*). Their intimate structure, on the other hand, was quite uniform. The nuclei were large and round or oval, with a definite vesicular appearance. The chromatin was mostly condensed at the periphery of the nucleus, forming a distinct nuclear membrane. There were cells with several large nucleoli, and often two nuclei were seen. The surrounding cytoplasm was rather scarce, with short amphophilic processes, which had a tendency to fuse with similar processes of adjacent cells. At times the cytoplasm was homogeneous, dark and compact; at other times it was palely stained and finely vacuolated owing to small droplets of fat, as revealed by the sudan stain. In addition to these cells, the predominant element in the growth, a moderate number of giant multinucleated cells resembling the Sternberg-Reed cells were seen. Lymphocytes and spindle-shaped cells with elongated vesicular nuclei were irregularly scattered, singly or in small groups. The latter, identified as fibroblasts, were chiefly seen in proximity to blood vessels, which, though not numerous, completed the histologic picture of the tumor. The vessels in longitudinal and cross section were mostly blood capillaries with a monostratified layer of endothelial cells lying on a fine fibrous membrane. With the exception of a few areas showing thin bundles of connective tissue fibers, in most of the examined areas no intercellular material could be seen, the cells of the growth appearing closely packed together. Sections impregnated with silver (Foot method) revealed a network of delicate fibers which surrounded almost every cell (fig. 2 *C*). This network of argentaffin fibers was not homogeneous in distribution but was abundant in some areas, scarce in others and at times completely absent. In the areas in which the vacuolated fat-containing cells were most numerous the argentaffin reticulum was also most prominent.

Where the infiltration of tumor cells into the myocardial tissue was most pronounced, the myofibrils showed damage. In many zones they were completely missing; in others they were widely spread apart by the tumor cells and atrophied, with loss of cross striations and thinning of nuclei.

These features were exactly reproduced by the polypoid formations bulging into the cavity of the right auricle and invading the right auricular appendix. The same picture was shown by the enlarged periaortic and peribronchial lymph nodes. The usual lymph-glandular structure was completely obscured by the diffuse neoplastic invasion.

COMMENT

In order to exclude any other possible source of sarcoma in the body, a thorough study was made of representative sections from the principal organs. Special attention was devoted to the prostate, which grossly was enlarged, and to the mass on the neck diagnosed as lipoma. The sections from the prostate revealed no evidence of cancer but rather a generalized hyperplasia affecting both stroma and glands. The mass on the neck was definitely lipoma. The primary source of the cardiac tumor was established beyond question.

Outstanding histologic features of the tumor were: (*a*) a diffuse distribution of cells of a rather large polygonal type with a pale vesicular nucleus surrounded by scarce cytoplasm which often displayed peripheral amphophilic processes; (*b*) evidence of storage of a lipoid substance in many of these cells; (*c*) a thick network of argentaffin fibers in close relationship to the cells of the growth; (*d*) a moderate number of multinucleated giant cells, of lymphocytes and of spindle-shaped cells, the latter identified as fibroblasts.

A consideration of the cell types is of importance in understanding the genesis of the growth. The exact interpretation of multinucleated giant cells in a new growth offers difficulties. On the one hand is their frequency in Hodgkin's dis-

ease; on the other is the established fact that giant cells may be found in almost all types of cancer and at times also in noncancerous growths. Hodgkin's sarcoma, according to Gall and Mallory,[16] "retains the fundamental background of Hodgkin's lymphoma," the peculiar difference being the marked preponderance of the Sternberg-Reed cells over all other elements comprising the tumor. Although giant cells closely resembling the Sternberg-Reed type were present in the tumor, the diagnosis of Hodgkin's sarcoma was not warranted because the giant cells were inconspicuous and no other features of Hodgkin's disease were present. Predominant, on the contrary, were smaller cells which bore a close resemblance to immature mesenchymal cells. Some of these cells contained lipoid droplets. That this was a true storage of fat rather than cellular damage was shown by the good state of preservation of the cells and by the minute size of the fat droplets. The thick network of argentaffin fibers intimately related to these cells was another characteristic of the growth. In some areas the argentaffin fibers were numerous and surrounded almost every cell in individual patterns; in other areas no intercellular material could be seen. Roulet[17] similarly recognized among mesenchymal tumors which he thought had arisen from reticulum cells two varieties, one characterized by the presence of exceedingly numerous argentaffin fibers in close relation to the cells, the other poorer in fibers and chiefly composed of large cells fused together with loss of demarcation. To the former group, in his opinion, belong the more mature tumor types, to the latter the more immature ones. I have already accepted this division, for which the terms of "fibrillogenous" and "afibrillogenous" have been proposed.[18] In this case both fibrillogenous and afibrillogenous areas were found.

Among the characteristics of normal reticulum cells more frequently mentioned and ascribed to their growths are phagocytosis, mobilization and formation of argentaffin reticulum fibrils. Warren and Picena,[19] however, remarked that these properties are by no means pathognomonic of these tumors, for otherwise almost any neoplasm having a mesenchymal origin could be listed as reticulum cell sarcoma. The tendency of the newly produced cells to form syncytia-like reticular structures and the appearance of the nuclei, which "give the impression of being empty because of the striking contrast of the darkly stained nuclear membrane and the scanty and lightly stained chromatin," are regarded by them as diagnostic features that more properly agree with the criteria given by Oberling[20] in his original description of "réticulo-sarcome indifferencié." A nuclear pattern closely resembling the one described by Warren and Picena[19] in their cases of reticulum cell sarcoma was recognizable in the cells of the sarcoma now described. Nevertheless, but for a tendency of the cells to fuse together occasionally by means of short cytoplasmic bridges, no definite evidence of arrangement in synctium-like reticular structures was shown, most of the individual cells appearing entirely separated from each other.

On the basis of the presence or the absence of syncytial disposition of the tumor cells, Erlich and Gerber[21] have subdivided lymphosarcoma into reticular and intermediary types, the former characterized by homogeneous syncytial sheets, the latter by individual cells distinctly separated and closely conforming to the description given for the stem cell of the normal lymph node. In discussing this proposal, Gall and Mallory[16] noted the fundamentally similar cytologic characteristics of the two types of growths, sufficient to warrant their unification in a single histologic entity. As the tumor is made up of highly undifferentiated, presumably pluripotential cells, they propose the term "stem cell lymphoma." The principal characteristics of this cell type are, according to them, a pale-staining

17. Roulet, F.: Virchows Arch. f. path. Anat. **277**:15, 1930; **286**:702, 1932.
18. Tedeschi, C.: Arch. ital. di anat. e istol. pat. **6**:671, 1935.
19. Warren, S., and Picena, J. P.: Am. J. Path. **17**:385, 1941.
20. Oberling, C.: Bull. Assoc. franç. p. l'étude du cancer **17**:259, 1928.
21. Erlich, J. C., and Gerber, I. E.: Am. J. Cancer **24**:1, 1935.

amphophilic cytoplasm possessing a poorly defined outline and at times intercellular bridges, a large nucleus with extremely delicate chromatin, and a prominent vesicular nucleolus. The features described and illustrated in the Gall and Mallory publication closely resemble the intimate features of the growth which I have described, for which a similar genetic interpretation is suggested.

Considering that cells similar in all respects to the primordial undifferentiated cells of the lymph-hemopoietic system have been described by some (Ferrata [22]) in all mesenchymal structures, including the heart (Tedeschi [23]), I conclude that in this case also the matrix of the tumor may be found in an atypical proliferation of this type of undifferentiated mesenchymal cell.

Definite proof has been presented of the multiple developmental potentiality of the mesoderm comprising the celomic "mesothelium." These potentialities, shown by embryologic and experimental evidence,[24] were advocated by some to explain the wide morphologic variations of pleural [25] and pericardial tumors.[26] The possibility of an origin of the tumor in this case in mesothelial pericardial cells can be, however, ruled out, as no evidence of atypical proliferation of mesothelial cells could be seen in either of the pericardial layers. The histologic evidence also excludes the question of a source of the neoplastic cells in the myocardial fibrils, as none of the cellular shapes characteristic of a growth from myofibrils were seen.

Among the primary cardiac neoplasms described in the literature there is none the histologic description of which is similar to that of the one reported here. The closest resemblance is seen in certain features of the 2 neoplasms observed by Choisser and Ramsay,[15] notably the presence of embryonic cells, lymphoid elements and well organized reticulum; however, in the present case the neoformation of blood vessels was not so pronounced as to justify the diagnosis of angioreticuloendothelioma. The well established fact that the right auricle is by far the most common site of origin of cancerous cardiac growths is corroborated by the case illustrated here.

Additional interest in the case is derived from the discrepancy between the inconspicuous clinical findings and the extensive cardiac neoplasm. The patient did not have demonstrable evidence of any cardiac trouble until the last period of life, in which the symptoms were those of rapid cardiac decompensation. As possible reasons for the frequent absence of serious cardiac dysfunction in patients with extensive neoplastic involvement of the heart, Geipel [27] assumed that the slow development of the growth might give time for compensatory changes, whereas Bardenheuer [28] expressed the opinion that in most of the cases it is due mainly to the fact that the valves are free from invasion. Weller [14] stressed the frequent respiratory symptoms in patients with cardiac tumors, and in the case of Norton [29] this was so marked as to simulate an infection of the upper respiratory tract. Despite the extreme narrowing of the pulmonary orifice, the expected respiratory distress was not apparent in the case. Physical signs from change of posture have been described in cases of ball valve thrombus; symptoms of this kind were not shown by the patient, however, although anatomic conditions able to bring about a similar condition were present. Sudden occlusion of the tricuspid orifice by the penetration of the intracavitary pedunculated tumor masses may account, very likely, for the sudden death.

22. Ferrata, A.: Le emopatie, Milan, Soc. Ed. Libr., 1918, vol. 1.
23. Tedeschi, C.: Boll. Soc. med.-chir. di Modena **35**:373, 1935.
24. Marchand, F.: Beitr. z. path. Anat. u. z. allg. Path. **69**:1, 1921. Cunningham, R. S.: Bull. Johns Hopkins Hosp. **33**:257, 1922; **35**:111, 1924. Maximow, A.: Arch. f. exper. Zellforsch. **4**:1, 1927.
25. Klemperer, P., and Rabin, C. B.: Arch. Path. **11**:385, 1931. Klemperer, P., and Tedeschi, C.: J. Mt. Sinai Hosp. **8**:710, 1942.
26. Natali, C.: Cuore e circolaz. **10**:195, 1926.
27. Geipel, P.: Centralbl. f. allg. Path. u. path. Anat. **10**:846, 1899.
28. Bardenheuer, F. H.: Centralbl. f. allg. Path. u. path. Anat. **34**:337, 1923-1924.
29. Norton, W. H.: Am. J. M. Sc. **158**:689, 1919.

SUMMARY

Primary sarcoma of the heart arose from the right auricle in a 68 year old white man. The outstanding histologic features of the tumor were (*a*) a diffuse distribution of highly immature mesenchymal cells, which at times showed evidence of storage of a lipoid substance; (*b*) a thick network of argentaffin fibers in close relation to the cells of the growth; (*c*) a moderate number of multinucleated giant cells, of lymphocytes and of spindle-shaped elements. Evidence of metastatic involvement was found in a few periaortic and peribronchial lymph nodes. The clinical symptoms were those of rapid cardiac decompensation and sudden death.

EXTRAGENITAL CHORIOMA: ITS RELATION TO TERATOID VESTIGES IN THE TESTICLES

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Though the first report of chorioma of the male appeared in 1878,[1] it was not until 1902 [2] that the tumor was identified as similar to chorioma of the female. These and later contributions [3] soon made evident that the neoplasm was essentially primary in the testicle and in most if not all cases represented one-sided development of teratoma.[4] The first intimation of possible primary extragenital origin of chorioma was voiced in 1903.[5] Other reports followed,[6] but the first to be accepted by all critics did not appear until 1929.[7] Within the next thirteen years there were added to the literature reports of 8 cases of presumably bona fide extragenital chorioma.[8] But even of these some were questioned by later students [8b] who contended that the contributing authors had failed to report serial histologic study of the testes—such study being the only acceptable criterion for the exclusion of tumor primary in this source. The assumption of so critical an attitude had its inception with Prym,[9] who in a case of chorioma seemingly of extragenital origin discovered after careful scrutiny a small scar in one testicle. This he interpreted as evidence of a previously existing but then degenerated primary tumor of the testicle. Eight years later Craver and Stewart [10] reported a somewhat similar experience: that of a large tumor in the mediastinum and smaller ones in the pleura and the liver. Only after microscopic examination did they find in a testis a cyst lined by stratified squamous epithelium and a branching mucous gland with fully developed ducts. These authors cited as similar a third case, that of den Hartog,[11] in which a small pigmented area interpreted as retinal pigment was found in the testicle.

These 3 instances thus form the basis for the dictum that before a case can be considered as one of chorioma of extragenital origin the testes must be studied most carefully for presence of microscopic remnants of teratoid elements since these may serve as a source for metastasis.

It is our purpose to add to these a fourth case, in which a small cyst lined by stratified squamous epithelium was found in one testicle and widespread chorioma elsewhere.

From St. Vincent's Hospital.

1. Malasses, L., and Monod, C.: Arch. de physiol. norm. et path. **5**:375, 1878.

2. Schlagenhauffer: Wien. klin. Wchnschr. **5**:209, 1902.

3. Cooke, J. V.: Bull. Johns Hopkins Hosp. **26**:215, 1915. Handfield-Jones, R. M.: Brit. J. Surg. **13**:608, 1926. Ross, J. M.: J. Path. & Bact. **35**:563, 1932. McDonald, S., Jr.: Am. J. Cancer **34**:1, 1938.

4. Steinert, H.: Virchows Arch. f. path. Anat. **174**: 232, 1903. Taylor, M. L.: J. Path. & Bact. **14**:144, 1910. Levi-Valensi, J.; Montpellier, J.; Debrie, M., and Bartholi, M.: Semaine d. hôp. de Paris **11**:179, 1935.

5. Ritchie, J.: J. Obst. & Gynaec. Brit. Emp. **4**:65, 1903.

6. Frank, R. T.: J. A. M. A. **46**:248, 1906 [case 3]. Askanazy, M.: Verhandl. d. deutsch. path. Gesellsch. **10**:58, 1906. Bonney, V.: Tr. Path. Soc. London **58**: 9, 1907. Fischer, W.: Arb. a. d. Geb. d. path. Anat. **6**:358, 1908. Nakayama: Gann **4**:5, 1910-1911. Lambert, S. W., and Knox, L. C.: Tr. A. Am. Physicians **35**:17, 1920. Miller, J., and Browne, F. J.: J. Obst. & Gynaec. Brit. Emp. **29**:48, 1922.

7. Krasnianskaya, P. V.: Mosk., med. j. **9**:1, 1929; cited by Kantrowitz.[8d]

8. (*a*) Arendt, J.: Fortschr. a. d. Geb. d. Roentgenstrahlen **43**:728, 1931. (*b*) Heaney, H. G.: Am. J. Cancer **19**:22, 1933. (*c*) Fenster, E.: Frankfurt. Ztschr. f. Path. **46**:403, 1934. (*d*) Kantrowitz, A. R.: Am. J. Path. **10**:531, 1934. (*e*) Gerber, I. E.: J. Mt. Sinai Hosp. **2**:135, 1935. (*f*) Mathieu, A., and Robertson, T. D.: Internat. Abstr. Surg. **69**:158, 1939. (*g*) Weinberg, T.: Am. J. Path. **15**:783, 1939. (*h*) Erdmann, J. F.; Brown, H. A., and Shaw, H. W.: Urol. & Cutan. Rev. **45**:1, 1941.

9. Prym, P.: Zentralbl. f. allg. Path. u. path. Anat. **49**:98, 1930.

REPORT OF A CASE

A. M., a 37 year old white man, entered St. Vincent's Hospital, in New York, April 11, 1943, with a chief complaint of backache. This had begun insidiously six weeks before, was at first limited to the lumbar region and later radiated down both thighs. During this period he noted the loss of 20 pounds (9 Kg.). Another symptom, present for a week or two, was severe sweating, bearing no relation to the time of the day, the temperature or the amount of bed clothing used. At times there were chills. A few days before admission a cough developed but no hemoptysis. An interesting phenomenon had been noticed for six weeks by the patient and for six months by his wife, namely, gradual enlargement of his breasts. Epigastric swelling and generalized pain of the joints were also reported.

Nodes were palpable in the inguinal regions. A soft systolic blow was heard over the apex of the heart. Palpation of the abdomen revealed a mass in the right upper quadrant, interpreted as an enlarged liver. Special attention was paid to the testicle, with negative results. The prostate gland did not appear enlarged.

The patient failed rapidly, becoming weaker and more prostrate. He suffered chiefly from severe backache and pain in the right flank. On April 20 icterus appeared and gradually deepened. Toward the end of the month two nodules in the left testicle became palpable. This was a new development, for previously, despite careful examination of the testicles at regular intervals, nothing was found. Throughout, the patient was febrile, the temperature ranging from 99 to 101 F. Death occurred May 6, about ten weeks after the onset of symptoms.

10. Craver, L. F., and Stewart, F. S.: J. A. M. A. **106**:1802, 1936.

11. den Hartog, B. J. C.: Chorionepithelioma in Male and Its Biologic Significance, Thesis, Amsterdam, J. H. de Bussy, 1933, p. 60; cited by Craver and Stewart.[10]

The urine of this patient revealed nothing abnormal. The blood showed a hemoglobin content estimated to be 66 per cent of normal (9.5 Gm.) and 3,510,000 red cells and 6,600 white blood cells per cubic millimeter. The differential white cell count was 69 per cent polymorphonuclear leukocytes, 29 per cent lymphocytes and 2 per cent monocytes. The Kahn test of the blood was negative. Examination of sputum for tubercle bacilli by smear and Ziehl-Neelsen stain showed none. Blood cultures were sterile. Roentgen study disclosed many shadows interpreted as metastatic tumor in both fields.

Autopsy.—The body was normally developed but thin. The configuration of the body and the distribution of hair were normally male. No abnormal changes were discernible in the external genitalia. The breasts were slightly enlarged. From both a thin fluid could be expressed. The liver was markedly enlarged, the inferior margin reaching the pelvic brim. The organ weighed 4,590 Gm. Scattered through it were large hemorrhagic friable nodules measuring up to 5 cm. in diameter. Nodules were also found throughout both lungs. A moderately large, roughly spherical encapsulated tumor, weighing 539 Gm. and measuring 10 by 10 by 7.5 cm., occupied the left retroperitoneum. It extended downward from the inferior surface of the left renal vein, to which it was adherent. Laterally the growth covered the lower half of the kidney, while medially it reached the vertebral column. The capsule was thin. On section the tumor appeared solid, friable and hemorrhagic, with a few areas soft and gray. The prostate gland was diffusely enlarged, measuring 5.5 by 3 by 4 cm. and weighed 49 Gm. Adenoma of a lateral lobe and subcervical adenoma were observed, the tumors being 1 cm. in diameter. The seminal vesicles appeared normal.

The right testicle weighed 28 Gm. and appeared natural. The left was small, weighing 10 Gm. It con-

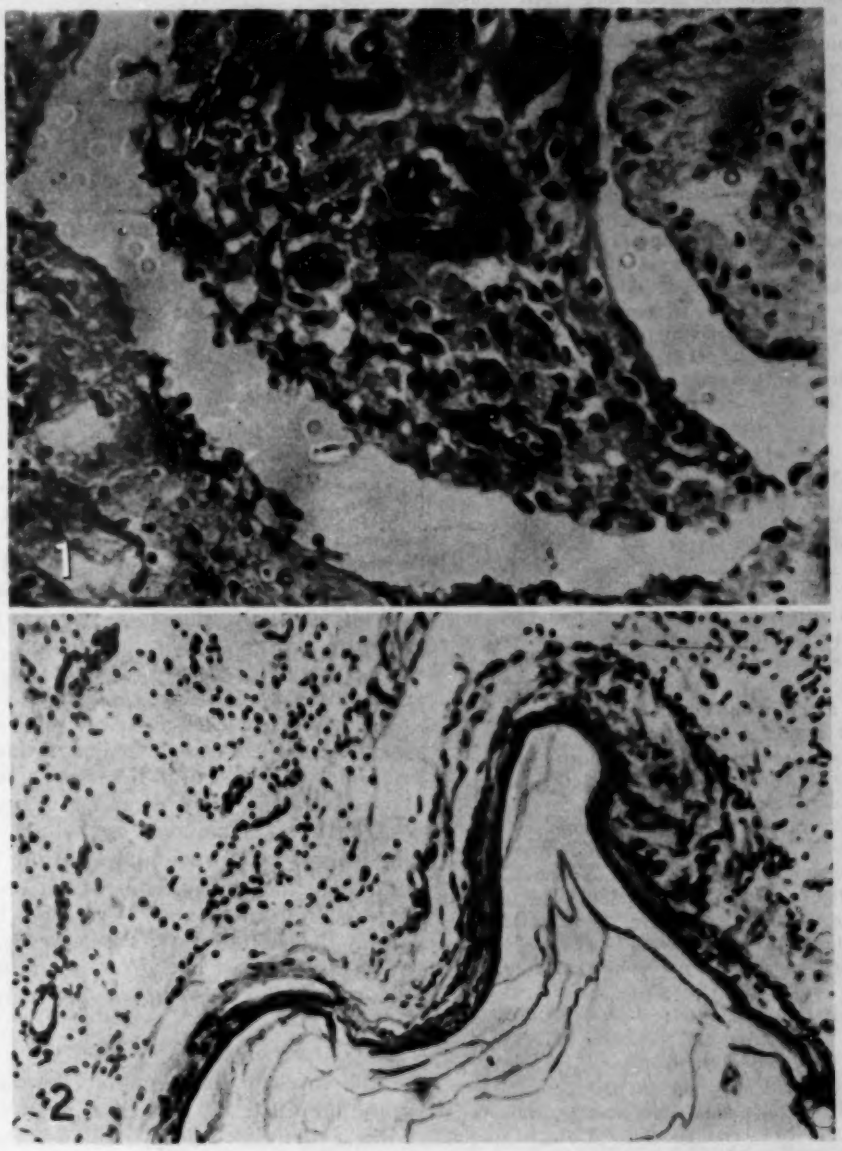

Fig. 1.—High power microscopic view of chorioma invading a blood channel.

Fig. 2.—Low power view of the cyst in the left testicle. Note the keratin in the lumen. The lining epithelium is much flattened out but is stratified squamous in type. The surrounding testicular tubules are atrophied.

tained a small scar, 1 to 2 mm. in diameter. On the outer surface of the tunica vaginalis six raised nodules, 10 to 15 mm. in diameter, were present—solid, friable and hemorrhagic. These were in no way in contact with the testis proper, being separated from the tunica albuginea by the space between the parietal and the visceral layer of the tunica vaginalis. The heart, the kidney, the spleen and the pancreas were normal. The right adrenal gland weighed 6.9 Gm. and the left 8 Gm., both appearing normal. The thyroid gland was small, firm and natural in appearance. No unusual changes were noted in the brain. The pituitary gland weighed 0.5 Gm. and grossly appeared normal.

The gross anatomic diagnosis was: chorioma of retroperitoneal origin with metastases to the lung, liver and tunica vaginalis; atrophy of the left testicle; noncancerous adenoma of the prostate gland; gynecomastia.

Microscopic Examination.—The tumor had the same histologic appearance in all sites. The neoplastic cells were exceedingly pleomorphic. Particularly conspicuous were large multinucleated cells with abundant eosinophilic cytoplasm; others were also large but were mononuclear, the nucleus having a bizarre appearance. Still other cells were smaller, with more uniform nuclei. The cells were variously arranged—usually in small clusters and at times in larger irregular masses. Often the tumor elements were found surrounding large, irregular sinusoidal channels. Not infrequently they appeared within the lumens of these channels, free or attached to the surface (fig. 1). Striking were the extensive necrosis and hemorrhage throughout the tumor.

The tubules of the prostate gland were dilated and lined by narrow, relatively short columnar cells with basal nuclei and scant cytoplasm. No secretory activity was evident. The adenomatous lateral lobe and subcervical area contained the same type of tubules. In the medial portion of the adenoma was found vascular fibromatous tissue of the type believed to be the precursor of glandular adenoma. The seminal vesicles were normal.

In the testes a few tubules had a normal appearance. Many, however, were smaller, were lined by sustentacular cells and contained only a few spermatogonia. Some also showed fibrous thickening of the basement membrane. There was no hyperplasia in Leydig cells. The left testicle was more atrophic, containing many completely fibrosed tubules. Others not fibrosed were devoid of all except sustentacular cells. Numerous large islands of Leydig cells were scattered throughout. In the area of scar noted grossly there was a small cyst, approximately 0.25 mm. in diameter, lined by a narrow layer of stratified squamous epithelium and containing a small amount of keratin (fig. 2).

The cortex of the adrenal gland was thin owing to atrophy of the glomerular and reticular layers. The fascicular layer appeared normal.

The pituitary gland was cut in half, and sections were prepared from each half. No attempt was made to study the gland by serial section. Though special stains were employed, no differential count was attempted. Examination of these sections as reported by Dr. Aura Severinghaus disclosed diminution in and degranulation of acidophil cells, also increase, degranulation and vacuolation of basophil cells. The kidney, spleen, pancreas, brain and thyroid gland were essentially normal.

The breast disclosed hyperplasia of ducts and periductal connective tissue. The connective tissue immediately adjacent to the ducts was loose; that more distant, dense.

COMMENT

Chorioma of the male has been adequately discussed in the literature from both the clinical and the pathologic point of view. Regardless of whether the tumor is primary in the testicle or arises elsewhere, the clinical picture is the same: that of a rapidly growing neoplasm. Once symptoms develop, the decline of the patient is rapid. Grossly and microscopically the tumor is characteristic, identical with chorioma of the female.

Many males with chorioma exhibit gynecomastia. Our report merely furnishes another example of this. The basic change responsible for the enlargement of the breasts is the increase in the ducts and periductal connective tissue. Some authors also find proliferation of acini. This was not observed in our case.

The pituitary gland has been described as normal in some cases, while in others presence of pregnancy cells and vacuolation of basophils were found. In our case, both these changes were observed and interpreted as indicating the presence of excess estrogen.[12] Identical alterations are seen in the female.

The significance and causation of the atrophy of the adrenal cortex in the case reported are puzzling unless it is part of the general emaciation.

A further noteworthy finding was an atrophic left testicle with hyperplasia of Leydig cells—a phenomenon often associated with atrophy of testicular tubules.[13] In the literature are two other instances of the discovery that one testicle [8b] or both [8a] were atrophied. This phenomenon might well be related to hypersecretion of estrogen, the effect of which could be augmented further by the destruction and dysfunction (jaundice) of the liver in our case.[14] Prostatic hypertrophy, present in our case, has been noted by other authors [15] but is not a constant finding. Of particular interest, and the reason for presenting this paper, was the discovery that one testis contained a small cyst lined by stratified squamous epithelium. This finding suggests a previously existing more active teratoma, metastasis from which resulted in the extragenital chorioma. That this type of neoplasm degenerates in its primary site while its metastases persist and grow is admittedly only a theory, which cases such as ours may support.

SUMMARY

The cases of extragenital chorioma described in the literature fall into three groups: (1) those with a definite, easily demonstrable testicular tumor, (2) those in which small teratoid vestiges are found in the testis and (3) those in which careful examination of the testes by serial histologic section reveals neither. The case reported by us belongs to the second group.

12. Severinghaus, A.: Personal communication to the author.

13. Jemerin, E. E.: Arch. Surg. **35**:967, 1937.

14. Morrione, T. G.: Paper read at the meeting of the New York Pathological Society, Oct. 28, 1943.

15. Kantrowitz.[8d] Mathieu and Robertson.[8f]

Notes and News

University News.—Promotions at Washington University, St. Louis, include that of Margaret G. Smith to associate professor of pathology and that of William O. Russell and that of Paul A. Wheeler to assistant professor. Samuel H. Gray has been appointed associate professor of pathology.

Justin R. Dorgeloh has been appointed assistant professor of pathology in the University of Southern California.

W. A. Derrick has resigned as associate professor of pathology in the University of Virginia to become director of the city laboratory of Kingston, N. Y.

S. E. Sulkin, instructor in bacteriology and immunology, Washington University, St. Louis, has been placed in charge of the virus research laboratory of the medical school of the Southwestern Medical Foundation, Dallas, Texas.

Armin Von St. George, associate professor of pathology at the New York University School of Forensic Medicine and since 1916 assistant director of the laboratories at Bellevue Hospital, died on November 20. He was 51 years old.

Grants for Research in Endocrinology.—Requests to the National Research Council Committee for Research in Endocrinology for aid during the fiscal period from July 1, 1944 to June 30, 1945, will be received until February 28. Application blanks may be obtained by addressing the Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, Washington, D. C. In addition to a statement of the problem and the program, the committee desires information regarding the proposed method of attack, the institutional support of the investigation and the uses to be made of the sum requested.

Society News.—The next meeting of the American Society of Clinical Pathology will be held in Chicago June 8, 9, 10 and 11, 1944, with the Drake Hotel as the headquarters.

The American Academy of Pediatrics has given the Mead Johnson awards for 1943 to Hattie E. Alexander for her work on the treatment of Haemophilus influenzae infections and to Philip Levine for his work on erythroblastosis fetalis and the Rh factor.

Books Received

PATHOLOGICAL HISTOLOGY. Robertson F. Ogilvie, M.D., F.R.C.P. (Edinburgh), lecturer in pathology and assistant in forensic medicine, University of Edinburgh, and senior pathologist, Royal Infirmary, Edinburgh. Foreword by A. Murray Drennan, M.D., F.R.C.P. (Edinburgh), professor of pathology, University of Edinburgh. Edition 2. Price $9. Pp. 411, with 235 photomicrographs in color. Baltimore: The Williams & Wilkins Company, 1943.

The first edition received favorable notice in the ARCHIVES (**40**:841, 1940). The second edition is equally praiseworthy with respect to the illustrations, which are the distinctive feature of the book. The number of illustrations has increased from 220 to 235, all original photomicrographs in color, and almost without exception they represent faithfully and clearly the microscopic appearances of well prepared stained sections. The staining methods, mostly by far hematoxylin and eosin, and the magnification are given in each case. The text has been "completely revised" and increased by 76 pages, but the book remains essentially a good atlas of pathologic histology with explanatory notes. The text is not without faults. To mention just one: The statements about the seasonal incidence and the portals of infection of epidemic poliomyelitis are not in accord with present knowledge. There are 411 pages of text, preface and index and about 120 leaves of special paper on which the photographs are reproduced. With two exceptions (figs. 90, 91 and 92; figs. 139, 140 and 141), just one side of each leaf is used, frequently only in part, which means that at least some 120 pages are blank.

CARNEGIE CORPORATION OF NEW YORK. REPORT OF THE PRESIDENT, THE SECRETARY AND THE TREASURER FOR THE YEAR ENDED SEPTEMBER 30, 1943. Pp. 115. New York: Carnegie Corporation of New York, 1943.

MICROSCOPIC TECHNIQUE IN BIOLOGY AND MEDICINE. E. V. Cowdry, professor of anatomy, Washington University, and director of research, the Barnard Free Skin and Cancer Hospital. Pp. 206. Price $4. Baltimore: The Williams & Wilkins Company, 1943.

This book contains a great deal of valuable information about microscopic technic. The presentation is concise. The references are included in the text. Topics are arranged alphabetically on double column pages. The chapters on choice of methods, on laboratory organization and on standardization of stains are highly instructive. The book should be available in every laboratory in its field.